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Publication date:
2018

Document Version
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Citation (APA):
Gussmann, M. K. (2018). *Development of a herd- and cow-specific decision support tool for control of mastitis*. DTU Veterinærinstituttet.

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DEVELOPMENT OF A HERD- AND COW-SPECIFIC DECISION SUPPORT TOOL FOR CONTROL OF MASTITIS

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PhD thesis
Lyngby, June 2018

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This thesis was written with LaTeX using classicthesis v4.6.

Mathematics is a place where you can do things
which you can't do in the real world.

— Marcus du Sautoy

PREFACE AND ACKNOWLEDGEMENTS

Before I moved to Denmark, I was working as a research assistant at the Friedrich-Loeffler-Institut's Epidemiology Section in Germany. Sometime in the beginning of 2015, one of my co-workers had a guest from Denmark. Apparently, they had two open PhD positions in modelling at DTU. In our section, there were two mathematicians, both working with modelling, whose contracts were soon running out—Jana and me. So the guy, Tariq, suggested that we should apply. That was how both of us ended up as PhD students at DTU Vet.

I want to thank Tariq for this suggestion and, more importantly, for being a great supervisor during the three years of my PhD. A big thank you also to my other supervisors Nils and Søren for their help and input on my way to becoming a PhD.

As part of my PhD, I was at Utrecht University in January and February 2018. I want to thank Wilma Steeneveld for her help with my last two papers and the epidemiology group in Utrecht for welcoming me there. I also want to thank the other two members of our little mastitis working group at DTU Vet: Carsten Kirkeby for the model planning and coding sessions (I still think we should recode the model to clean it up and make it faster, don't you?) and Lisa Zervens for helping out whenever I had a question about the more practical aspects of dairy cows.

My PhD project was part of the STOPMAST project, which was funded by the Danish Milk Levy Foundation (Mælkeafgiftsfonden). Thank you to the other STOPMAST members, especially Line Svennesen for taking me on my first dairy farm visit and Michael Farre for helping out with specific knowledge about mastitis and mastitis treatment in Denmark.

Godelind and Camille, even though the social events had a bumpy start, it was fun planning them together, thank you for that. And to all my colleagues in the epidemiology groups here at DTU Vet—thank you for the last three years, you made working here a great experience!

But the last three years were not all about work. A month after my arrival, I started learning Danish. Even though two evenings a week became a bit much after a while, we had a lot of fun in class. Or after class on Wednesdays for beers. Or on our trip to Sweden. Or on many other occasions. Thank you all, and I hope we will continue to have fun together! And a big thank you, Jamie—also for taking the time and reading my thesis.

I met Sabrina at one of these thematic lectures for PhDs at DTU (good thing I was wearing the "Counting sheep" shirt that day). We became good friends, and Sabrina and Nico took me mountain biking and flying in the air tunnel. I didn't think I'd end up liking that, so thank you both for taking me! Also... we should get back to our cake project, Nico.

And finally, there's my friends from work—thank you for everything, brunches, coffee, tea, cake, parties... Thank you also for our nice trips together, Carolina, for our writing sessions towards the end, Ana, and thank you Patrik and everyone else who supported me in finishing my thesis.

Maya Lyngby, 29. June 2018

SUMMARY

Mastitis, or intramammary infection (IMI), is one of the most frequent diseases in dairy cattle. In addition to being painful for the affected cow, especially in the case of a clinical mastitis, it also has various other effects on production and herd routines. Mastitis not only reduces the milk yield but also the milk quality. It disrupts the herd routine by leading to increased culling rates. Furthermore, clinical cases have to be treated. The milk loss and the costs for necessary control and intervention lead to considerable economic losses for the dairy farmer.

An important element of mastitis management is antimicrobial treatment. In view of rising antimicrobial resistance, the use of antibiotics in production animals has garnered the concern of consumers and politicians. Mastitis prevention and control strategies should therefore be multifaceted and only rely on antibiotics when necessary.

The overall objective of this PhD project was to identify such multifaceted and cost-effective intervention strategies. The project itself was divided into two parts.

The first part of this PhD project focused on data analysis. Register data from the Danish Cattle Database were analysed by herd-wise logistic regressions for determinants for antimicrobial treatment in relation to udder health. Principal component analysis and clustering were performed on the regression coefficients to group herds according to their treatment patterns. Lactational treatments and dry cow treatments were considered separately throughout the whole data analysis.

The results showed that in both cases, herds grouped according to the most prominent determinants. Treatment was determined by milk production, age, or diagnostics for dry cow treatments. For lactational treatments, the determinants were milk production, age and diagnostics, or whether or not a cow was subsequently culled.

In the second part of the PhD project, a strain-, cow-, and herd-specific bio-economic simulation model of intramammary infections was developed and used to investigate and compare different mastitis intervention strategies. The model incorporates a previously existing model of a Danish dairy herd (iCull). It additionally simulates the spread of several mastitis pathogens within the herd, the effects of mastitis, and intervention measures for clinical and subclinical cases.

The developed model simulates a Danish dairy herd with several mastitis pathogens, and the transmission framework is strain- and cow-specific: it specifically allows simulating different strains of the same pathogen species, and it considers cow-specifics for infection and cure. However, careful calibration to specific herd conditions is paramount as the model is sensitive to changes in the transmission parameters.

The modelled interventions included antibiotic treatment and cow-specific reactive culling of infected animals for both clinical and subclinical cases. Some intervention measures also partly incorporate the findings of the first part of the project. The investigated intervention strategies were divided into strategies against clinical mastitis and strategies against both clinical and subclinical mastitis.

The most effective strategy against clinical intramammary infections was a "good hygiene", represented by a low transmission rate. However, specifics about the necessary measures to achieve "good hygiene" and the costs associated with implementing such measures are unknown. Cost-effectiveness, on the other hand, could be improved by using more antibiotic treatments or by culling. More specifically, a comparison of different intervention strategies showed that cow-specific treatment or culling decisions for clinical cases was most cost-effective. For these intervention strategies, the number of antibiotic treatment days was reduced at the cost of an increased number of culled cows.

When the intervention strategies against clinical mastitis were supplemented by cow-specific treatment or culling of subclinical cases, cost-efficiency could be further increased. Subclinical cases were identified by two subsequent high somatic cell counts ($\geqslant 200\,000\,\mathrm{cells/ml}$) and diagnostic testing for confirmation. Depending on the main causative pathogen and among

the investigated intervention strategies, the choice of the clinical intervention measure varied. For *Staphylococcus aureus*, the choice of the intervention measure against clinical cases affected cost-effectiveness (more cost-effective interventions before addition of measures against subclinical cases stayed more cost-effective after addition of such a measure). For *Streptococcus agalactiae*, which primarily causes subclinical mastitis, the intervention measure against clinical cases was less relevant.

The preferred intervention strategy therefore depends on the herd. It may also vary depending on the farmer's preferences regarding management measures such as treatment or culling. Intervention strategies against mastitis should therefore be herd-specific.

The limitations of a modelling approach, as it was taken in this thesis, lie in the proper understanding of the transmission dynamics and its parameterisation. If parameter estimates are missing, for example in the case of hygienic measures, the respective aspects cannot be properly investigated. Factors that could not be included in the model, because of missing parameter values or because they were unknown, may change the outcome in a real life situation. Therefore, the model results should be seen as recommendations for possible cost-effective intervention strategies against mastitis until, ideally, they can be validated by field studies.

In conclusion, the model presented in this thesis can be used as a decision support tool in scientific research: it can identify cost-effective intervention strategies against mastitis, while also taking into account other related factors (e.g., antibiotic treatment and culling). These findings may then be considered in the future when planning new mastitis management strategies.

SAMMENDRAG

Yverbetændelse (mastitis), eller infektion i yveret, er en af de hyppigste lidelser hos malkekvæg. Mastitis er smertefuldt for dyrene, specielt ved klinisk mastitis, og påvirker mælkeydelsen, mælkekvaliteten og rutinerne i besætningen. Rutinen i besætningen påvirkes ved at øge udsætningsraten og antallet af kliniske behandlinger. Den reducerede mælkeydelse samt behovet for øget kontrol med mastitis giver desuden et økonomisk tab. Et vigtigt element i mastitis kontrol er behandling med antibiotika. I lyset af stigende problemer med resistens, har anvendelsen af antibiotika til produktionsdyr gjort forbrugerne og politikerne bekymrede. Forebyggelses- og kontrolstrategier bør derfor være alsidige og kun inkludere antibiotika når det er nødvendigt. Det overordnede formål med dette ph.d.-projekt var at identificere andre og omkostningseffektive interventionsstrategier. Projektet blev opdelt i to dele.

Den første del af projektet fokuserer på dataanalyse. Registerdata fra kvægdatabasen blev analyseret med logistisk regression for at afdække betydende faktorer for behandling med antibiotika i forbindelse med yversundhed. Principal Component Analyse og Clusteranalyse blev udført på regressionskoefficienterne for forskellige besætningsgrupper ud fra deres behandlingsmønstre. Laktationsbehandlinger og goldningsbehandlinger var dominerende i hele dataanalysen. Resultaterne viste for begge analyser at besætningerne kunne grupperes i forhold til de mest dominerende faktorer. For goldkøer blev behandlingerne bestemt af køernes mælkeproduktion, alder eller diagnostik. For laktationsbehandlinger var de vigtigste faktorer mælkeproduktion, alder og diagnostik, uanset om koen senere blev udsat.

I anden del af ph.d.-projektet blev en stamme-, ko- og besætningsspecifik bio-økonomisk simuleringsmodel for mastitis udviklet og brugt til at undersøge og sammenligne forskellige mastitis interventionsstrategier. Modellen bygger på en eksiste-

rende model af en dansk mælkekvægsbesætning (iCull). Modellen simulerer spredningen af adskillige mastitis patogener i en besætning, virkningerne af mastitis samt management strategier til at forhindre kliniske og subkliniske tilfælde. Modellen simulerer både specifikke stammer af mastitis patogener og er desuden ko-specifik med hensyn til infektion og helbredelse. Det er altafgørende at kalibrere modellen nøjagtigt til en specifik besætning, da modellen er følsom overfor ændringer i spredningsparametrene. De modellerede interventioner inkluderede antibiotisk behandling og ko-specifik udsætning af inficerede dyr af både kliniske og subkliniske tilfælde. Nogle kontrolforanstaltninger benytter desuden nogle af resultaterne fra første del af projektet. De modellerede interventionsstrategier var grupperet i strategier mod klinisk mastitis og strategier mod både klinisk og subklinisk mastitis. Den mest effektive strategi mod klinisk mastitis var "god hygiejne" repræsenteret ved en lav spredningshastighed. Der mangler dog kendskab til de simulerede og nødvendige foranstaltninger for at opnå "god hygiejne". Desuden er omkostningerne af sådanne foranstaltninger ikke kendt. Omkostningseffektiviteten kan forbedres ved at anvende flere antibiotiske behandlinger eller udsætning. En sammenligning af forskellige interventionsstrategier viste at kospecifikke behandlingsbeslutninger var de mest omkostningseffektive for kliniske tilfælde. Her blev antallet af antibiotiske behandlingsdage reduceret mens antallet af udsatte køer blev øget.

Når interventionsstrategierne mod klinisk mastitis blev suppleret med kospecifik behandling eller udsætning af køer med subklinisk mastitis, kunne omkostningseffektiviteten øges yderligere. Subkliniske tilfælde blev identificeret ved to på hinanden efterfølgende høje målinger af SCC (Somatic Cell Count, $\geqslant 200\,000\,\mathrm{celler/ml}$) som efterfølgende blev bekræftet diagnostisk. Valget af interventionsforanstaltningen mod kliniske tilfælde blev påvirket af patogenet: Tiltag mod subkliniske tilfælde af Staphylococcus aureus blev mere omkostningseffektive ved at kombinere dem med andre tiltag; for Streptococcus agalactiae, som primært forårsager subklinisk mastitis, var interventionsforanstaltningen mod kliniske tilfælde mindre relevant. Den foretrukne interventionsstrategi afhænger derfor af besætningen og dens situation. Den kan også variere afhængigt af landbrugerens

præferencer med hensyn til forvaltningsforanstaltninger som behandling eller udsætning. Interventionsstrategier mod mastitis bør derfor være besætningsspecifikke.

Begrænsningerne af en modelleringsmetode som beskrevet i denne afhandling ligger i forståelsen af spredningsdynamikken og dens parametrisering. Hvis der mangler parameter estimater, f.eks. omkring hygiejniske forhold, kan dette ikke undersøges korrekt. Faktorer som ikke kunne medtages i modellen på grund af manglende parameterværdier eller fordi de var ukendte kan ændre resultatet i en reel situation. Derfor bør modelresultaterne ses som gode indikationer for mulige omkostningseffektive interventionsstrategier mod mastitis indtil de ideelt set kan valideres.

For at konkludere, kan modellen i denne afhandling anvendes som et beslutnings støtteværktøj i videnskabelig forskning: Den kan identificere omkostningseffektive interventionsstrategier mod mastitis, samtidig med at der tages hensyn til andre relaterede faktorer (f.eks. antibiotikabehandling og udslip). Disse resultater kan inddrages i fremtidige overvejelser når man planlægger nye mastitis managementstrategier.

ZUSAMMENFASSUNG

Masitits ist eine Entzündung der Milchdrüse, meist verursacht durch Bakterien, und ist eine der am häufigsten auftretenden Krankheiten bei Milchkühen. Sie verursacht Schmerzen, vor allem in klinischen Fällen, und beeinträchtigt zusätzlich den landwirtschaftlichen Betrieb: Die Milchproduktion und Milchqualität erkrankter Kühe ist reduziert, Schlachtungsraten sind vergleichsweise hoch und klinische Fälle müssen behandelt werden. Die Milcheinbußen und zusätzlichen Kosten für notwendige Kontrollmaßnahmen oder Interventionen führen zu großen wirtschaftlichen Nachteilen für den Bauern.

Ein wichtiges Element bei der Prävention und Kontrolle von Mastitis ist die Behandlung mit Antibiotika. Die Verwendung von Antibiotika in der Tierhaltung gerät in Anbetracht zunehmender Antibiotikaresistenz jedoch verstärkt ins Bewusstsein und in die Kritik von Verbrauchern und Politik. Strategien zur Prävention und Kontrolle von Mastitis müssen daher heutzutage verschiedene Herangehensweisen in Betracht ziehen und können sich nicht nur auf Antibiotika beschränken.

Das Ziel dieses PhD-Projekts war es, kosteneffiziente Interventionsstrategien zu finden, die sich nicht nur auf die Behandlung mit Antibiotika konzentrieren.

Der erste Teil des PhD-Projekts beschreibt die Datenanalyse. Registerdaten der dänischen Rinderdatenbank (kvægdatabasen) wurden auf bestimmende Faktoren für Antibiotikabehandlungen des Euters oder im Zusammenhang damit analysiert. Dabei wurde zwischen Behandlungen während der Laktationsperiode und bei der Trockenstellung unterschieden. Die Analyse wurde für jede Behandlungsart einmal durchgeführt: Für jede Herde wurde zunächst Antibiotikabehandlung durch eine logistische Regression modelliert. Anschließend wurden eine Hauptkomponentenanalyse und eine Clusteranalyse durchgeführt.

Die Ergebnisse zeigen, dass bei beiden Behandlungsarten die Herden in drei Gruppen unterteilt werden können. In beiden

Fällen war Milchproduktion einer der bestimmenden Faktoren. Desweiteren wurde für Behandlungen bei der Trockenstellung jeweils eine Gruppe durch Alter bzw. diagnostische Ergebnisse charakterisiert. Während der Laktationsperiode waren diese beiden Faktoren gemeinsam für eine Gruppe bestimmend; in der dritten Gruppe war mit entscheidend, ob die erkrankte Kuh im Nachfolgenden geschlachtet wurde.

Im zweiten Teil des PhD-Projekts wurde ein biologisch-ökonomisches Simulationsmodell entwickelt, das durch Bakterien verursachte Mastitis simuliert. Das Modell baut auf einem zuvor beschriebenen Modell einer dänischen Milchkuhherde (iCull) auf und simuliert darüberhinaus sowohl die Ausbreitung von mehreren verschiedenen Mastitiserregern in der Herde als auch die durch Mastitis hervorgerufenen Effekte und verschiedene Maßnahmen gegen klinische und subklinische Mastitis.

Das entwickelte Modell lässt ausdrücklich verschiedene Stämme einer Bakterienart zu, und sowohl Infektion als auch Heilung sind von Besonderheiten der jeweiligen Kuh abhängig. Das Modell ist damit bakterienstamm-, tier- und herdenspezifisch. Demnach muss es sorgfältig für die jeweilige Herde kalibriert werden, da selbst kleine Änderungen in einigen der Modellparameter die Ergebnisse beeinflussen können.

Schließlich wurde das Modell dafür verwendet, verschiedene Interventionsstrategien zu untersuchen und zu vergleichen. Die implementierten Strategien beinhalten sowohl die Behandlung mit Antibiotika als auch die Schlachtung von infizierten Kühen für klinische oder subklinische Mastitis. Einige der Interventionsmaßnahmen ziehen außerdem Ergebnisse aus dem ersten Teil des Projekts in Betracht. Die Interventionsstrategien wurden unterteilt in klinische Strategien, die ausschließlich Maßnahmen gegen klinische Mastitis enthielten, und kombinierte Strategien, die darüber hinaus Maßnahmen gegen subklinische Mastitis einbezogen.

Unter den untersuchten klinischen Strategien ist „gute Hygiene“, repräsentiert durch eine niedrige Infektionsrate, am effektivsten. Welche spezifischen Maßnahmen notwendig sind, um eine gute Hygiene zu erreichen, und die damit verbundenen Kosten sind jedoch nicht ausreichend untersucht, weshalb

Kosteneffektivität nicht festgestellt werden konnte. Verbesserte Kosteneffectivität konnte dafür durch den Mehrgebrauch von Antibiotika oder durch mehr Schlachtungen erreicht werden. In den kosteneffektivsten Interventionsstrategien wurde fallspezifisch zwischen einer Behandlung mit Antibiotika oder Schlachtung entschieden. Dies reduzierte die Verwendung von Antibiotika, erhöhte allerdings die Anzahl an geschlachteten Kühen.

Durch Ergänzung der klinischen Maßnahmen mit Maßnahmen gegen subklinische Mastitis konnte die Kosteneffizienz in den kombinierten Strategien weiter verbessert werden. Subklinische Fälle wurden dabei durch zwei aufeinanderfolgende hohe Zellgehalte in der Milch ($\geqslant$200 000 $\mathtt{Zellen/ml}$) identifiziert und durch einen diagnostischen Test überprüft. Abhängig davon, welcher Erreger hauptsächlich auftrat, spielte die jeweilige gewählte klinische Maßnahme eine mehr oder weniger wichtige Rolle: Bei *Staphylococcus aureus* führten kosteneffizientere klinische Maßnahmen zu kosteneffizienteren kombinierten Strategien, während bei *Streptococcus agalactiae*, welcher hauptsächlich subklinische Mastitis verursacht, die klinische Maßnahme keine größere Rolle spielt.

Die zu bevorzugende Interventionsstrategie hängt demnach von der jeweiligen Herde ab. Sie wird aber auch von den Präferenzen des Bauern abhängen, also davon, ob dieser lieber Antibiotika verwendet oder ob er bereit ist, mehr Kühe zu schlachten. In jedem Fall sollten Interventionsstrategien gegen Mastitis herdspezifisch gewählt werden.

Die vorgestellte Herangehensweise durch Modellierung bietet die Möglichkeit, verschiedene Interventionsmaßnahmen und -strategien unter gleichen Bedingungen zu untersuchen und zu vergleichen, ohne aufwändige und teuere Feldstudien durchführen zu müssen. Sie hat jedoch auch gewisse Einschränkungen und Grenzen: Zum einen ist zur Modellerstellung Wissen über die Verbreitungsdynamik der Krankheitserreger notwendig und zum anderen müssen die richtigen Parameterwerte bekannt sein. Wenn Parameterwerte fehlen, wie zum Beispiel im Fall von guter Hygiene, können die betroffenen Aspekte nicht oder nicht vollständig untersucht werden. Darüber hinaus können Wissenslücken dazu führen, dass relevante Faktoren nicht berücksichtigt

werden. Fehlende Parameterwerte oder Faktoren können dazu führen, dass sich in der Realität unerwartete Ergebnisse einstellen, die sich von den modellierten Ergebnissen unterscheiden. Die hier gefundenen Ergebnisse sollten deshalb in erster Linie als gute Leitindikatoren für mögliche kosteneffiziente Interventionsstrategien gegen Mastitis gesehen werden, bis sie im späteren Forschungsverlauf idealerweise bei Feldstudien bestätigt werden können.

Das in dieser Dissertation vorgestellte Simulationsmodell kann als Werkzeug zur Entscheidungshilfe in der wissenschaftlichen Forschung verwendet werden, da es kosteneffiziente Interventionsstrategien gegen Mastitis und damit verbundene Faktoren wie die Behandlung mit Antibiotika oder Schlachtungen identifiziert. Die gefundenen Erkenntnisse können in Betracht gezogen werden, wenn neue Präventions- und Kontrollmaßnahmen gegen Mastitis geplant werden.

CONTENTS

1 PURPOSE AND OUTLINE

The purpose of this PhD study was to improve mastitis control through identification of cost-efficient intervention strategies against mastitis in dairy cows, i.e., intervention strategies that can reduce the occurence of mastitis in a dairy herd without lowering the net income. The plan was to achieve this by developing a strain-, cow-, and herd-specific bio-economic simulation model that is able to simulate population and infection dynamics in a dairy herd. The model should then be used to investigate costs and efficacy of different pathogen-, cow- and herd-specific intervention strategies, showing that it can be used as a decision support tool in scientific research.

The PhD thesis itself is based on four manuscripts and has the following structure:

THE INTRODUCTION in Chapter 2 gives an overview over mastitis and modelling. In Section 2.3, the objectives of the PhD project and related research questions are presented.

CHAPTER 3 includes Manuscript I, in which data from the Danish Cattle Database are analysed for predictors of antimicrobial treatment in relation to udder health.

CHAPTER 4 presents the model (Manuscript II), and intervention strategies against clinical mastitis (Manuscript III), as well as combined intervention strategies against both clinical and subclinical mastitis (Manuscript IV).

AN OVERALL DISCUSSION of these manuscripts follows in Chapter 5.

THE CONCLUSIONS in Chapter 6 answer the research questions presented in Section 2.3.

CHAPTER 7 presents future perspectives.

Objective 1: Investigate if data from the Danish Cattle Database can be used to characterise different farmers' mastitis management

RQ1A, RQ1B

Manuscript I:
Determinants of antimicrobial treatment for udder health in Danish dairy cattle herds

Objective 2: Develop and test a strain-, cow-, and herd-specific simulation model of IMI

RQ2

Manuscript II:
A strain-, cow-, and herd-specific bio-economic simulation model of intramammary infections in dairy cattle herds

Objective 3: Investigate costs and efficacy of various intervention strategies against IMI

RQ3, RQ4, RQ5

Manuscript III:	**Manuscript IV**:
Economic and epidemiological impact of different intervention strategies against clinical mastitis	Economic and epidemiological impact of different intervention strategies against subclinical and clinical mastitis

Figure 1: Overview over the objectives, the manuscripts included in the thesis, and the related research questions (RQ, see Section 2.3).

2 INTRODUCTION

2.1 MASTITIS

Literally, mastitis means "related to the breast", coming from Ancient Greek mastós (breast) and -îtis (pertaining to), though the suffix -itis is now commonly used to indicate a disease or an inflammation. The word mastitis consequently describes an inflammation of the breast or the mammary gland and can, theoretically, occur in all mammals.

In this thesis, however, I will restrict all considerations to mastitis in dairy cattle, where it has an important economic impact (Halasa et al., 2007).

2.1.1 Biology

In dairy cattle, mastitis is also commonly called intramammary infection (IMI). While both terms are often used interchangeably, they are not exactly the same: IMI is an infection and mastitis is an inflammation of the mammary gland (Lopez-Benavides et al., 2012).

Intramammary infections are caused by infecting microorganisms, most often bacteria, e.g., *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus agalactiae*, or *Escherichia coli*. Therefore, IMI can usually be diagnosed by a suitable bacterial culture (Lopez-Benavides et al., 2012) or polymerase chain reaction (PCR) (Taponen et al., 2009). Typically, bacterial culture is the preferred method (Lopez-Benavides et al., 2012), as it detects live microorganisms, while PCR detects DNA of bacteria that are not necessarily viable.

An inflammation, on the other hand, describes foremost an immunological response, which is most often caused by infecting agents (Harmon, 2001; Lopez-Benavides et al., 2012). This response leads to an influx of leukocytes into the tissue of the

mammary gland, from where they can then infiltrate the milk (Harmon, 2001). Hence, mastitis is usually accompanied by a high somatic cell count (SCC). Clinical mastitis cases show visible signs in the milk (e.g., flakes or clots) or udder (e.g., swelling, heat, or pain). If there are no visible symptoms of inflammation, the mastitis is called subclinical and usually diagnosed by SCC or the California Mastitis Test (Lopez-Benavides et al., 2012).

In the following, when I talk about mastitis, it will assumed to be caused by an infection. I will also use the terms subclinical and clinical for IMI in the same way as they would be used for mastitis.

Occurrence

For a long time, *Streptococcus agalactiae* was considered to be one of the primary mastitis pathogens (Dodd et al., 1969; Murphy, 1956; Smith et al., 1985), with *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Escherichia coli*, and some *Klebsiella* species (Harmon, 1994; Smith et al., 1985; Zadoks et al., 2011) as other common major mastitis pathogens.

However, over the years, the relative impact of different mastitis pathogens has changed, probably due to the introduction of control programs and subsequent changes in prevalences (Pitkälä et al., 2004; Wilson et al., 1997). This led, for example, to common occurrence of coagulase-negative staphylococci (CNS) and *Corynebacterium bovis* (Pitkälä et al., 2004; Wilson et al., 1997), though these are considered minor pathogens, as their impact seems to be lower than that of the major pathogens (Schukken et al., 2009; Taponen and Pyörälä, 2009).

Among all these mastitis causing pathogens *S. aureus* may be the most studied (Zadoks et al., 2011). Although this could also be connected to its importance as a human pathogen (Zadoks et al., 2011), *S. aureus* appeared as the most prevalent isolate, e.g., in a study conducted by Østerås et al. (2006). Still, it is important to remember that there can be large variations in prevalence between different herds (Piepers et al., 2007). To my knowledge there are, however, no recent studies about the distribution of different mastitis pathogens on cow level in Denmark.

For *S. agalactiae* in particular, this change over time resulted in decreased within-herd prevalences in the 1990s (Keefe, 1997). In

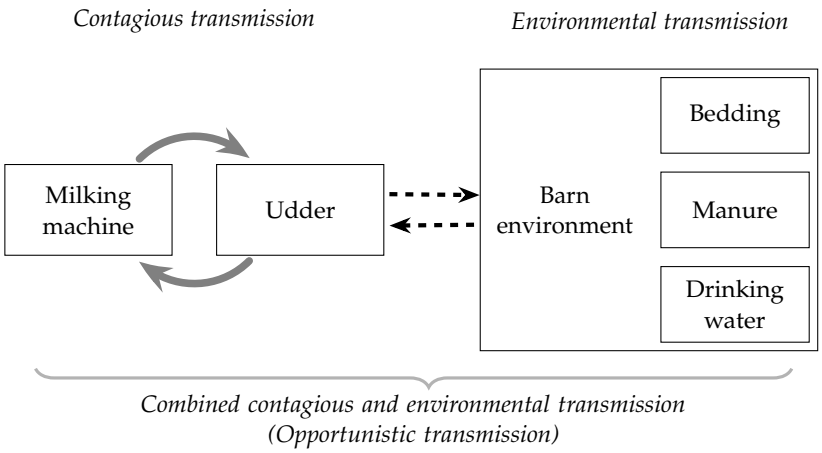

Figure 2: Transmission routes of mastitis pathogens.

Denmark, herd level prevalence of *S. agalactiae* decreased from over 30% in the 1950s to about 2% in 1992 (Mweu et al., 2012) until, starting in 2000, the number of infected herds began to increase again (Mweu et al., 2012). In 2017, approximately 5.5% of the herds were tested positive (Farre, 2017). A similar development could be seen in other Scandinavian countries (Jørgensen et al., 2016). On top of that, formerly efficient control of *S. agalactiae* was shown to no longer be as effective, indicating a change in transmission behaviour (Jørgensen et al., 2016).

Transmission

There are two major reservoirs for mastitis pathogens in a dairy herd: the infected udder and the herd environment, e.g., bedding or manure (Harmon, 1994; Smith et al., 1985).

Bacteria from an infected udder can spread during the milking process (Harmon, 1994) by remaining on or in parts of the milking equipment, from where they can be transferred to other quarters. For instance, fluctuations in the milking vacuum might cause a reflux of milk from a contaminated milking claw to the teats (Besier et al., 2016). This transmission route is contingent on the udder as a reservoir and, therefore, depends upon the number of infected quarters. In contrast, new infections with bacteria from the herd environment seem to occur primarily between milkings (Harmon, 1994) and are thought to be independent of the incidence (Zadoks

et al., 2001a). These two transmission routes are commonly called contagious and environmental transmission, respectively.

Historically, every mastitis pathogen was categorized as either contagious or environmental: *S. aureus* and *S. agalactiae* were considered contagious, while other pathogens were seen as environmental (Harmon, 1994; Smith et al., 1985). However, in more recent times, this strict distinction has been disputed. Zadoks et al. (2001a) found that a model describing contagious transmission seemed to fit their data better than one for environmental spread, even though *S. uberis* was thought to be an environmental pathogen. Moreover, molecular studies of various pathogens suggest that contagious or environmental behaviour may not be inherent to a species but rather differ between strains (Zadoks et al., 2011): if several cows in a herd are infected by a single strain, one would assume cow-to-cow spread. Contrarily, strain heterogeneity for one pathogen species in a herd suggests environmental transmission. Furthermore, Jørgensen et al. (2016) found that *S. agalactiae*, which was formerly considered a strictly contagious pathogen (Murphy, 1956), exhibited parallel contagious and environmental transmission cycles: in addition to contagious transmission through the milking machine, *S. agalactiae* could be found in the barn environment, in bovine faeces, and in the drinking water, i.e. in typical environmental samples (see Figure 2).

Consequently, mastitis pathogens should not be classified into contagious and environmental based on their species but rather depending on the specific strains present in a dairy herd (Schukken et al., 2012). Additionally, a third transmission type combining the basic contagious and environmental transmission routes may be considered.

Risk factors

Transmission does not only depend on the causative pathogen. Various other factors have been investigated for facilitating establishment of IMI. Zadoks et al. (2001b) found, among other factors, that age, lactation stage, a previous IMI, and a high SCC were cow or quarter level risk factors for mastitis. Steeneveld et al. (2008) also identified lactation stage, previous IMI, and SCC, as well as season as risk factors for clinical mastitis.

Other studies linked a high herd milk yield to an increased clinical mastitis incidence (Schukken et al., 1990; Syväjärvi et al., 1986) or found an unfavourable genetic correlation between clinical mastitis and milk yield (Koeck et al., 2014; Syväjärvi et al., 1986). Yet, actually estimating the extent to which milk production influences mastitis is more difficult (Seegers et al., 2003). Furthermore, milk flow (or milking speed) has been considered as a risk factor for IMI. In this case, however, the evidence seems to be contradictory. Jensen et al. (1985) reported a positive genetic correlation between milk flow and clinical mastitis, whereas Miller et al. (1978) could not find evidence for an association between faster milking and increased mastitis risk. Besier et al. (2016) suggested that it could be a combination of several milking parameters that are responsible for an increase in mastitis occurence.

Biological effects on production

While a high herd milk yield seems to be a risk factor for mastitis, mastitis in return reduces the milk production of the individual cow.

Several studies have shown that clinical mastitis reduces the milk yield of the affected cow until the end of the lactation (e.g., 110 kg to 552 kg over the entire lactation, Rajala-Schultz et al., 1999). Houben et al. (1993) even found that this effect could carry over into the next lactation, if a cow had three or more cases of clinical mastitis. The amount of milk lost seems to depend on the causative pathogen (Gröhn et al., 2004; Hertl et al., 2014b) and the number of previous clinical mastitis cases of a cow in a lactation (Hertl et al., 2014b).

Similarly, subclinical mastitis can be associated with a reduced milk production: mastitis is generally accompanied by a high SCC, as described above, and elevated SCC levels lead to a reduced milk yield (Hagnestam-Nielsen et al., 2009; Halasa et al., 2009a; Hortet et al., 1999). In this case, too, the causative pathogen influences the milk loss; studies have shown that different pathogens generally differ in how much they affect SCC (Schukken et al., 2009; Wilson et al., 1997).

Other studies have suggested that clinical mastitis could also alter a cow's heat cycle (Moore et al., 1991) or reduce the probability of conception (Barker et al., 1998; Hertl et al., 2014a).

2.1.2 Interventions

In 1969, Dodd et al. wrote that they

> ...cannot conceive of any system of completely preventing all udder disease; therefore, the object of a control must be to reduce udder infection to a low level.

They proposed three possibilities for reducing IMI: increasing cure rates, replacing infected cows, or reducing infection rates. This is still the case today.

Lactational treatment

Increasing the cure rates should naturally decrease the prevalence, as fewer cows will remain infected. In the case of contagious pathogen strains, it can also be expected that fewer new infections will occur as a consequence (Steeneveld et al., 2011), which would further contribute to reducing infection.

To increase cure rates for IMI, infected quarters (intramammary treatment) or cows (systemic treatment) can be treated. Lactational treatment is most commonly antimicrobial intramammary treatment (Barkema et al., 2006), although systemic administration of antibiotics may be beneficial for *S. aureus* mastitis and is recommended for severe *E. coli* cases (Pyörälä, 2009). In addition, it can be supplemented by the use of non-steroidal anti-inflammatory drugs (NSAIDs), mostly for pain relief (Breen, 2017). Different treatment strategies may have different cure rates also depending on cow characteristics (Steeneveld et al., 2011). Cure probabilities may also differ between two different strains of the same species (van den Borne et al., 2010b). In any case, milk from animals receiving antibiotic treatment must not be sold until the risk of finding antibiotic residue in the milk is low (Edmondson, 2014). This withdrawal period may be around 6 to 7 days, but it depends on the antibiotic used and the method of administration.

In Denmark, clinical IMI are typically treated with antibiotics or, in some cases, the cow is culled. Treatment is usually administered intramammarily. Subclinical IMI do not commonly receive lactational treatment.

Dry cow treatment

Another type of treatment is dry cow therapy (DCT). DCT consists of antibiotic treatment of cows at dry off, i.e., after the last milking in the lactation. It is aimed at increasing the cure rates as well as reducing the infection rate in the dry period by preventing new infections (Dodd et al., 1969).

There are two forms of DCT, blanket DCT (all cows are treated) and selective DCT (only some cows are treated). Selective DCT and blanket DCT are both similarly effective in curing infections, however, treating all cows is generally better for preventing new infections (Rindsig et al., 1978).

Selective DCT originated from economical considerations (Morris et al., 1978), but today, concern about antimicrobial resistance is another factor in favour of selecting cows for selective DCT (Halasa et al., 2009c). In Denmark, blanket DCT is not allowed and cows need to have a positive diagnostic result (often using PCR) not older than 42 days to receive DCT. Another possibility to determine infection status of cows for DCT was used by Scherpenzeel et al. (2016), who used different thresholds for high SCC as indicators for infection.

Culling

Similar to the increased cure rates described above, replacing infected animals with uninfected ones will decrease prevalence immediately, and subsequently it should lead to less new infections with contagious pathogen strains.

Replacement of infected animals implies that the farmer has to dispose of them in some way. As these cows are diseased, this will usually happen by culling the cows in question. For instance, Milian-Suazo et al. (1988) concluded that there were three major reasons for culling dairy cows: low milk production, poor reproductive performance, and udder problems, all of which can be related to mastitis (see Section 2.1.1). Others have found

that mastitis increased the culling risk for affected cows (Gröhn et al., 1998; Piepers et al., 2009). These findings suggest that farmers may actually use culling as a control measure. However, in scientific literature, culling is seldom considered as an intervention but rather as a side effect of IMI (Halasa and Hogeveen, 2018). Most studies of culling strategies in connection with mastitis investigate premature culling in the more general scope of optimal replacement strategies (Cha et al., 2014; Heikkilä et al., 2012), while only two studies included culling as an intervention measure (van den Borne et al., 2010a; Halasa, 2012).

Hygiene

Reduction in infection rates can be expected to lead to a lower prevalence — perhaps not immediately, but in the long term and with a lasting effect. Lowering the infection rate can therefore be considered more of a prevention than an intervention measure.

A high level of biosecurity can avoid introduction of new mastitis pathogens or pathogen strains into a herd (Barkema et al., 2009). Furthermore, mastitis pathogens depend on direct contact with contaminated material during milking or in the environment for transmission (see Section 2.1.1). For this reason, transmission of these pathogens is subject to the conditions, i.e. equipment and management, in each herd. Neave et al. (1966) found that following a simple hygiene routine at milking could reduce the number of new infections. In other studies, inadequate milking machine calibration and the associated irregular fluctuations in the milking vacuum could lead to increased mastitis prevalence (Besier et al., 2016; Nyhan and Cowhig, 1967).

Hygiene, proper milking machine maintenance, and biosecurity can therefore be expected to lead to reduced transmission or infection rates, and these measures are included in the ten point recommended mastitis control plan (NMC, n.d.).

There are, however, only few studies investigating the actual effect of hygienic measures on mastitis (Huijps et al., 2010; Lam et al., 1996; Neave et al., 1969).

2.1.3 Economics

As mentioned above, bovine mastitis, or IMI, is one of the most costly diseases in dairy cattle and it has a great economic impact on herd economics (Halasa et al., 2007). Costs arise from both interventions (see Section 2.1.2) and the described production effects (see Section 2.1.1):

If an IMI is treated with antimicrobials, it leads to additional costs for drugs, veterinary services, diagnostic testing, additional labour, and for discarded milk. Culled cows have to be replaced either by buying replacement stock or by raising new animals. In this case, further indirect costs could arise if the new cow has a lower milk yield than the culled cow (Halasa et al., 2007). On the other hand, milk production in cows with subclinical or clinical mastitis also leads to substantially less income for the farmer (Halasa et al., 2007).

Costs for mastitis are usually underestimated by the farmer (Huijps et al., 2008), and estimates can vary substantially. Halasa et al. (2009b), e.g., found costs between €101 and €328 per clinical case or between €0 and €310 per subclinical case of mastitis. In general, costs vary from one herd to another depending on various factors, for example causative pathogen (Halasa et al., 2009b), mastitis incidence, or management (Halasa et al., 2007). Therefore, an economically beneficial strategy in one herd might not be economically beneficial in another.

Within a herd, costs differ between cows depending for example on milk yield potential, age, or pregnancy status (Cha et al., 2014). Furthermore, as described in Section 2.1.1, risk factors (previous IMI, Hertl et al., 2014b) or the causative pathogen may also affect the impact on production and thus influence the costs. Hence, differences between cows are also taken into consideration when replacement strategies are evaluated: what is the benefit of keeping a cow versus replacing it? This is called retention payoff (RPO) (Cha et al., 2014).

As there are differences in costs between both herds and cows, economic decisions should take *pathogen-*, *cow-*, and *herd-specifics* into account. It should also be kept in mind that farmers may be equally motivated by economic factors and other perceived benefits, e.g., healthy animals (Valeeva et al., 2007). Jansen and Lam

(2012) suggested that there were two importanct factors determining mastitis management in a herd: the farmer had to believe that there was a mastitis problem and that a management strategy would be effective. Hence, in addition to economic benefits, effectiveness of intervention strategies should be communicated to farmers.

Yet, while cost-effectiveness has been studied for intervention strategies comprising treatment or culling (van den Borne et al., 2010a; Halasa, 2012), or single hygienic measures (Huijps et al., 2010), little is known about cost-effectiveness of comprehensive hygiene or biosecurity strategies.

One way to investigate and compare different intervention strategies is through a modelling approach, which leads to the second part of this chapter.

2.2 MODELLING

The first known mathematical model in epidemiology was published in 1766 by Daniel Bernoulli, showing the advantages of smallpox inoculation (Bernoulli and Blower, 2004). Since then, epidemiological models have developed and grown more complex. Today, we differentiate between different types of models, the most common being compartmental models, network models, and agent- or individual-based models (Lanzas and Chen, 2015). The modelled population is divided into groups or compartments, depending on their infection status, e.g., susceptible, infectious, and recovered.

In compartmental models, the individuals, or rather the units of interest, are not considered separately. Instead, a certain contact structure is assumed for the infection process. Then, transitions between the compartments are modelled and the compartment sizes are tracked. Network models are focused on more realistic contact structures. Agent-based models, on the other hand, explicitly model each individual and their characteristics (Lanzas and Chen, 2015). But what can these models be used for?

2.2.1 Models as decision support tools

Reality is complex. Pathogen transmission, e.g., can be influenced by a multitude of possibly interrelated factors (Lanzas and Chen, 2015), as described in Section 2.1. Models can be aimed at understanding this complexity by modelling assumed structures and interdependencies and investigating how they influence the model dynamics (Keeling and Rohani, 2008). A possibly more obvious use of models is for prediction (Keeling and Rohani, 2008). A predictive model that can be used to forecast the outcome of different decisions is, in a way, a decision support tool.

Predicitive models have to be as accurate as possible to be able to guide decision making (Keeling and Rohani, 2008). For that, they have to address the complexity, lest they guide decision making in the wrong direction (Lanzas and Chen, 2015). Hence, modellers usually have to defend why simplifications or assumptions in their model will still allow studying the respective research question. However, complexity can also lead to unforeseen results that may or may not match reality, so Basu and Andrews (2013) suggested that modellers should also be able to defend when a more complex model was needed, e.g., because of a strong *a priori* belief about a factor's influence.

The underlying structure of a model should be a good represention of reality, as "structural uncertainty" can have a bigger impact on model outcome than uncertainty in the parameter values (Basu and Andrews, 2013). Fitting a model to data, i.e. finding the "right" parameter values, is called model calibration. Basu and Andrews (2013) suggested that calibration could be used to filter out models or model structures that are not suitable, because they cannot be adequately fitted to data. Actual model validation is a difficult problem, considering that no two outbreaks are ever exactly the same, even if they were caused by the same pathogen (Heesterbeek et al., 2015). It is usually done by looking at the aggregate outcome of a model and comparing it to data or expectations until sufficient confidence in its validity is gained (Sargent, 2003). For this reason, it is particularly important, that models are well documented (Keeling and Rohani, 2008).

In summary, a good model should be built to be suitable for its purpose, incorporating just as much complexity as needed, and it should be able to be fitted to data (Keeling and Rohani, 2008). Still, no matter how complex or good the model is, it is important to remember that a model can never be expected to predict the future completely accurately (Keeling and Rohani, 2008).

Now, what are the requirements for a model as a decision support tool? The modeller has to understand the underlying structure of the problem, so they can program the model, and they need the data to parameterize it. The model has to be well described, so it can be understood and so that results are reproducible (for transparency); it has to be accurate, so as not to mislead decision making (accuracy); and it has to be flexible enough to be able to incorporate future changes or new information (flexibility) (Keeling and Rohani, 2008).

2.2.2 Modelling mastitis

It is a misnomer to talk about modelling mastitis, as it is not really mastitis but the underlying infection that is modelled. So, what is needed to model IMI in dairy herds? The first question should be the purpose of the model. In this work, I am interested in a decision support tool for prevention and control of IMI, as described in Chapter 1.

For that, I have to model a dairy herd including its main income, milk production, and IMI occurence and dynamics in the herd. Pathogen transmission should be included, because interrelations between transmission and interventions can be expected, at least for contagious pathogens (Section 2.1.2). As different pathogens can have different effects on the milk production, and two strains of one pathogen species may have different transmission behaviour (Section 2.1.1), the model should be *strain-specific*. It should also be *cow-specific*, to account for risk factors for IMI (Section 2.1.1) and differences in cure rates following treatment depending on cow characteristics (Section 2.1.2). To be able to investigate and compare different intervention strategies, options for treatment, culling, and DCT have to be included in the model. Furthermore, parameters like transmission rate depend

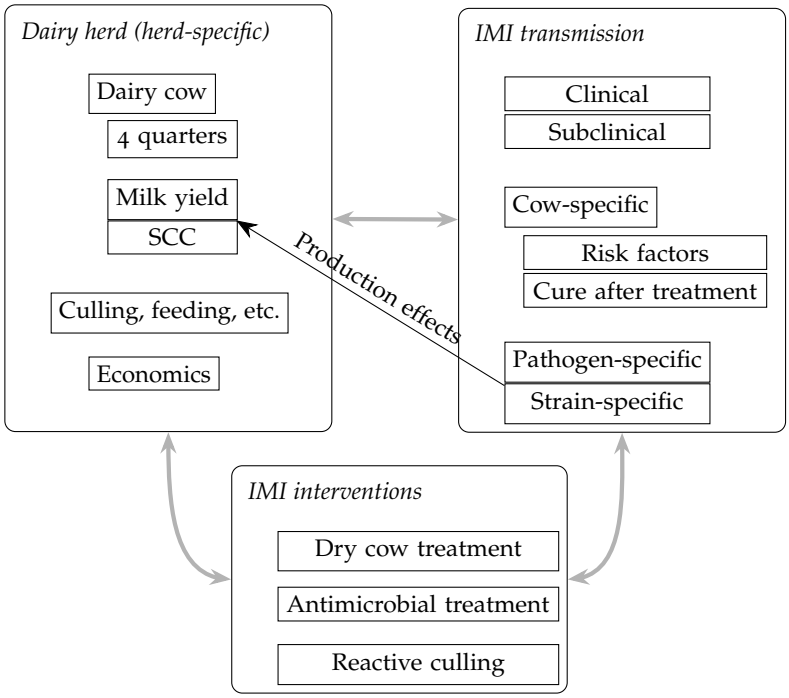

Figure 3: Overview over the requirements for a model used as decision support tool for prevention and control of IMI.

on conditions (hygiene, biosecurity) in a herd (Section 2.1.2) and farmers have different preferences regarding mastitis management (Huijps et al., 2009). Thus, *herd-specifics* should be taken into account. Including these elements (see Figure 3 for an overview) will allow investigation of costs and effectiveness of control and prevention strategies for mastitis considering realistic situations.

One of the first models for IMI was described by Dodd et al. (1969) with the aim to describe the dynamics of IMI in dairy herds. However, in the same study, Dodd et al. (1969) already suggested that mathematical models could be used in the future to investigate the effects of control strategies on mastitis. In later years, various models were developed to evaluate IMI control measures. Most of these models are stochastic and individual-based in the sense that individual cows and their disease states are explicitly modelled, though individual behaviour is not modelled and contact structures are simple.

In the following, I will introduce some of these bio-economic models, all of which include IMI transmission, as a comprehensive review of all models would need a chapter on its own.

SIMMAST Allore et al. (1998) developed a discrete-event simulation model, including contagious and environmental transmission. Infection depends on cow-specific parameters. Milk yield is modelled daily for every cow, and adjusted according to IMI status and pathogen. The model includes a treatment option for clinical cases, and a culling strategy for mastitis. This model was validated against data.

SIMHERD IV Østergaard et al. (2005) used SimHerd to model a typical Danish dairy herd in weekly time steps including, among other things, milk yield and SCC for every cow. IMI transmission is modelled through a baseline risk function, depending on the pathogen, lactation stage, and adjusted for cow risk factors. During the dry period, the baseline risk for clinical IMI is reduced. IMI cases are divided into different categories (subclinical, and mild, moderate, severe, or permanent effect clinical). This category determines, together with the pathogen, the effect on milk yield, SCC, feed intake, and body weight. Treatment of clinical cases leads to milk withdrawal for a number of days. There is a low probability that a new clinical case will be culled, otherwise IMI can indirectly lead to culling due to a reduced milk yield.

BOVINE IMI MODEL Halasa et al. (2009b) modelled contagious and environmental IMI in a Dutch dairy herd with a two week time step. Somatic cell scores are modelled for every cow and adjusted for IMI, a high bulk tank SCC leads to a penalty. Milk yield is also modelled for every cow and adjusted for production losses due to pathogen-specific clinical IMI or high SCC. Clinical cases are treated, and their milk is discarded for six days. Cows can be culled with a low probability due to clinical IMI, or, related to subclinical IMI, due to reduced milk production or conception failure. Replacement heifers are only introduced in case of a cumulative (milk) quota deficiency. Feeding costs are also adjusted for IMI. An extension of this model (Halasa et al., 2010) further includes IMI in the dry period. Transmission

during the dry period is based on separate parameters and always environmental; clinical cases can only occur in the first or last two weeks. In this extension, different options for DCT are included. A second extension (van den Borne et al., 2010a) adds treatment options for subclinical contagious IMI, and a third one introduces different treatment options for clinical IMI (Halasa, 2012).

While these models all have their strengths, they are also missing certain factors:

All three models simulate transmission on cow-level. Yet, infection and important interventions (e.g., treatment) happen on quarter-level. Modelling on quarter-level would allow up to four pathogens per cow and additional measures, e.g., drying off of single quarters. Furthermore, none of the models is strain-specific or includes cow-specific cure rates.

SIMMAST and the bovine IMI model only allow one pathogen per cow. The SIMMAST model is additionally missing IMI during the dry period and DCT. SimHerd, on the other hand, does not directly include contagious transmission, nor is DCT mentioned. It also does not consider culling as an intervention strategy against IMI. The bovine IMI model has no cow-specific infection and does not take pathogen-specific effects for subclinical IMI into account.

Hence, none of these models truly allow strain-, cow-, and herd-specific evaluation of prevention or intervention strategies against IMI for cost-effectiveness.

2.3 OBJECTIVES AND RESEARCH QUESTIONS

The general objective of this PhD study was to develop a strain-, cow-, and herd-specific model that could be used in scientific research to investigate costs and efficacy of different interventions against IMI (see Chapter 1), covering all points mentioned in Section 2.2.2.

To that end, it would help to understand how herds can differ in regards to mastitis management, i.e., how different farmers are dealing with mastitis in their herd. In Denmark, all cattle

related data is collected in the Danish Cattle Database. The first objective was therefore to *investigate, whether data from the Danish Cattle Database can be used to characterise different farmers' mastitis management*, specifically antibiotic treatment.

The second objective was to *develop and test a cow-, strain-, and herd-specific simulation model of IMI*. For that, an existing herd model (Kirkeby et al., 2016) was extended by adding a transmission framework for IMI, using literature values to parameterise the model. However, studies with parameter estimates are scarce and conditions are changing (see Chapter 4), so parameterization can be quite a challenge. Therefore, it is especially important to understand how the parameters influence the model.

Finally, the last objective was to *investigate costs and efficacy of various intervention strategies against IMI*. This objective was divided into two parts, *intervention strategies against clinical IMI* and *intervention strategies against both clinical and subclinical IMI*.

Figure 1 shows an overview over the objectives, how the corresponding manuscripts are connected, and where the following related research questions (RQ) fit.

RQ1A *Can already available data be used to determine and distinguish between treatment practices for mastitis in different herds?*

RQ1B *If so, what characterises the different groups/treatment practices?*

RQ2 *Which parameters are most influential in modelling spread of mastitis pathogens?*

RQ3 *Which intervention strategies against clinical IMI can reduce IMI incidence compared to a default three day intramammary antibiotic treatment and can an economic benefit be expected?*

RQ4 *Which intervention strategies combining interventions against both clinical and subclinical IMI can reduce IMI incidence compared to using only the clinical strategy? What are the economic effects?*

RQ5 *Is there a "best" intervention strategy against IMI?*

3 DETERMINANTS FOR TREATMENT

While the purpose of this PhD was to improve mastitis control through identification of cost-efficient intervention strategies by a modelling approach, it is also important to understand current practices in mastitis management.

Different approaches are conceivable to determine how farmers manage mastitis in their herd, e.g. interviews with farmers and veterinarians or farm visits. For this PhD project, another strategy was chosen: register data from the Danish Cattle Database were analysed for their value in relation to mastitis control. This approach, if successful, would have a notable advantage in that it could be easily repeated for updated data found in the Danish Cattle Database.

An important element in mastitis management is antimicrobial treatment, mostly of clinical cases or at dry off, as described in Section 2.1.2. In fact, mastitis is one of the main reasons for the use of antibiotics in Danish (DANMAP, 2016) and other European dairy herds (EMA and EFSA, 2017). However, consumer awareness regarding the use of antibiotics in the dairy industry is rising, asking for justifiable and responsible treatment strategies (Ruegg, 2003).

If data from the Danish Cattle Database could be used to characterise different farmers' mastitis management or, more specifically, antibiotic treatment strategies, this knowledge could be integrated into the second part of the PhD project (Chapter 4) to optimise antimicrobial usage in intervention strategies. Furthermore, understanding factors that determine whether a cow receives antibiotic treatment or not in a specific herd can aid in tailoring new management programs to the farmer's preferences.

This first part of the PhD project is presented in the first manuscript in Section 3.2 after a short presentation of the used methods.

3.1 MATERIALS AND METHODS

Data from the Danish Cattle Database (Section 3.1.1) were used as input in herd-wise logistic regressions (Section 3.1.2) to predict antibiotic treatment in the herds. Then, each herd, represented by a set of regression coefficients, was considered as a point in a sample space for principal component analysis (PCA, Section 3.1.3). The PCA transformed points were clustered to identify similar herds (Section 3.1.4). The overall predictive capabilities of the logistic regressions were evaluated by the area under the curve (AUC, Section 3.1.5).

3.1.1 Danish Cattle Database

The Danish Cattle Database is maintained by the Danish Agriculture & Food Council (RYK, 2016). It accumulates various cattle data, e.g., milk recordings, animal movements (entry and departure of animals), calvings, dry off, as well as animal health and treatment data. Entries into the database are made by farmers, advisory services, technicians, dairies, or slaughterhouses. Mandatory information is passed on to the Central Husbandry Register (CHR).

3.1.2 Logistic regression

Data from the Danish Cattle Database were used in mixed effects logistic regressions to predict antibiotic treatment for udder health reasons. A traditional approach might have used the possible predictors and herd as fixed effects, adding cow as a random effect, to assess probability for treatment under given circumstances. In this study (Section 3.2), we removed herd as an effect and ran one logistic regression for each herd instead. This allowed us to predict treatment for individual herds and compare between the different herds.

3.1.3 Principal component analysis

PCA is in essence a transformation (by rotation or reflection) of a sample space. If we assume that we have n variables and more observations than variables, then PCA can be seen as simply providing a new coordinate system. That is, the principal components are new coordinate axes for the sample space.

The principal components are chosen such that the first component accounts for the highest possible variation in point coordinates. The other components are added subsequently, catching as much variation in coordinates as possible under the constraint that they must be perpendicular to the prior principal components. This procedure ensures that there is more variation in the earlier principal components than in the later, while maintaining a cartesian coordinate system.

PCA is often used to reduce the dimension of data. The less variation there is in the last principal components, the better dimensionality reduction works. For example, if all observations are nearly the same in the last principal component coordinate, this coordinate could be ignored. PCA is not useful, on the other hand, if the observations are uniformly distributed in space.

3.1.4 Clustering

Clustering is the grouping of objects, so that obervations in one group are more similar to each other than to observations in other groups. There are a variety of clustering algorithms used in many different fields. In this study, we used a clustering algorithm devised by Ward (1963).

In Ward's algorithm, observations are grouped hierarchically, using the sum of the group error sum of squares as an objective function, where the error sum of squares of a group (ESS_{group}) with k elements is the sum of the squared differences between each observation x_i in the group and their mean value $\bar{x}$.

$$ESS_{group} = \sum_{i=1}^{k} (x_i - \bar{x})^2$$

The algorithm starts with n groups, one for each observation. The number of groups is then subsequently reduced by testing

all possible unions of two groups with respect to minimising the objective function until only one group including all observations is left.

3.1.5 Area under the curve

The diagnostic capability of any binary classifier or test that depends on a cutoff can be described by the receiver operating characteristics (ROC) curve. The ROC curve shows the relation between test sensitivity (true positive ratio) and test specificity (false positive ratio) and is therefore bounded by the unit square. It is plotted by varying the cutoff from its minimum to its maximum value and plotting sensitivity of the corresponding test over $1-$specificity. A perfect test would then position in the upper left corner $(0, 1)$, while the diagonal would describe random guessing. Therefore, tests should not have a ROC curve falling below the diagonal, as the diagnosis could be simply reversed to achieve a better result. A cutoff is usually chosen to minimise distance to $(0, 1)$.

The area under the ROC curve (AUC) is the integral over the ROC curve, i.e. the area between the ROC curve and the x-axis. It can be seen as a measure of how well the test performs over all possible cutoffs, although it does not specify for which range of cutoff values the test performs best. Generally, the AUC has a value between 0 ("always wrong") and 1 ("perfect test"), but, as described above, values below 0.5 can be avoided by reversing diagnoses for certain cutoff ranges.

3.2 MANUSCRIPT I

DETERMINANTS OF ANTIMICROBIAL TREATMENT FOR UDDER HEALTH IN DANISH DAIRY CATTLE HERDS

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Published in *Journal of Dairy Science* 101:505–517
https://dx.doi.org/10.3168/jds.2017-12994

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ABSTRACT

Societal pressure to limit the use of antibiotics in livestock production systems, including dairy cattle systems, is consistently increasing. To motivate farmers to reduce antibiotic usage, it is important to understand the factors that determine whether a cow will be treated with antibiotics or not. If farmers' usual practices regarding antibiotic treatments are taken into account, they may be motivated to adopt control measures that can facilitate prudent use of antibiotics and are at the same time cost-effective. In this study, we analyzed database recordings of milk yield and somatic cell count from the routine milk recording scheme, clinical registrations of mastitis and PCR results, and cow factors such as days in milk and parity in relation to antibiotic treatments for 518 dairy herds in Denmark. Farm-wise logistic regressions were used to predict antimicrobial treatment based on these factors. The resulting regression coefficients of 422 herds were further analyzed by principal component analysis and clustering to determine the driving predictors for treatment in different groups of farms. The results showed that determinants that were most important for predicting antibiotic treatments vary from one farm to another. Health indicators such as PCR or somatic cell count were most indicative for treatment on some farms, whereas other groups seemed to depend more on production factors (milk yield) or later culling of the cows. This shows that farmers behave differently and differences can be identified in register data. This information can be considered when developing costeffective herd-specific control measures of mastitis to promote prudent use of antibiotics in Danish dairy cattle farms.

KEY WORDS: dairy cattle, antibiotic treatment, mastitis, cluster analysis

INTRODUCTION

Mastitis is one of the most frequent and costly diseases in dairy cattle (e.g., Halasa et al., 2007). Besides impairing animal welfare (Broom, 1991; von Keyserlingk et al., 2009), it is also a major

reason for economic losses and prescription of antibiotics in dairy cattle herds (DANMAP, 2014, p. 34; EMA and EFSA, 2017, p. 29). The use of antibiotics in food animals has been a growing concern over the last decades, with increasing consumer awareness regarding this point and its effect on antimicrobial resistance (Ruegg, 2003).

Antimicrobial treatment is an important element in the management of mastitis in dairy herds. It is applied for treatment of clinical mastitis (Halasa, 2012; Steeneveld et al., 2011) and subclinical mastitis (van den Borne et al., 2010), and at dry-off to cure or prevent mastitis cases (Halasa et al., 2009a,b). However, its use must be prudent (i.e., limited to cases in which treatment with antibiotics is necessary while choosing a suitable antibiotic) to reduce the risk of antimicrobial resistance. To optimize antimicrobial usage, it is important to understand antimicrobial treatment patterns for udder health in dairy cattle herds and investigate factors that influence or enhance the treatments. However, it can be challenging to identify what farmers actually do, or why, as such information is not normally registered. Nevertheless, observable factors may give indications and thus may be useful as proxies for behaviors explaining antimicrobial treatment on a farm. Once influential factors are identified for a specific farm, veterinarians and udder health advisors can guide farmers to a prudent and cost-effective selection strategy of cows for treatment, while also taking the farmer's usual selection criteria or management practice into account. This might ease motivating farmers to adopt proposed management programs to improve udder health, thus aiding the prudent use of antimicrobials. As blanket dry cow therapy is prohibited in Denmark, an appropriate selection of cows for antibiotic treatment, both during lactation and at dry-off, is expected to have a positive effect on udder health and animal welfare while facilitating prudent use of antibiotics (Scherpenzeel et al., 2016). In Denmark, antimicrobials are prescribed by the herd veterinarian and exclusively distributed through pharmacies. In addition, treatments are normally carried out by veterinarians, but a farmer can have a herd health contract with a consulting veterinarian, allowing him to treat clinical cases of mastitis himself.

The proposed strategies can be developed and examined using, for instance, simulation models adjusted to the herd-specific parameters and with focus on cost effectively optimizing antimicrobial usage. These models can also consider other factors, such as spread of pathogens (e.g., van den Borne et al., 2010; Halasa et al., 2010), and thereby provide a more comprehensive understanding of management and treatment regimens and their expected outcomes, depending on given farm and cow parameters. This knowledge could additionally be used by policy makers when considering new regulations on a national scale.

In Denmark, herd and cow level registrations are collected in the Danish cattle database. They include, in addition to cow ID, for instance, milk yield and SCC from samples obtained through the routine milk recording scheme (6 or 11 times per year), and other recordings as part of a herd health scheme. The data also include recordings about diseases and treatments for individual cows and are being used for, among other purposes, the development of herd health and breeding programs. Its potential for development of herd-specific health management programs can, however, be further exploited.

We investigated whether data from the Danish cattle database could be used to predict antimicrobial treatment in relation to udder health management on different farms, and we identified differences between farms regarding treatment and determined which factors were most important for treatment on different farms. This information can be used to develop herd-specific strategies to improve udder health, considering prudent use of antimicrobials and the apparent selection strategy of cows for treatment.

MATERIALS AND METHODS

Data

Anonymized data from 1,500 randomly chosen conventional cattle farms with any milk yield recordings in Denmark, where at least 90 % of the animals are Danish Holstein cows, were retrieved from the Danish cattle database between February 27

and March 1, 2016. At this time, the total number of dairy farms in Denmark was 3,232. Data included information on milk yield, SCC, animal movements, reproduction and calving, dry-off dates, PCR results (from cow-milk samples), clinical registrations, and treatments. Clinical registrations are usually carried out by the veterinarian, but some farmers may also add to the registrations. These registrations include mainly the results of the California mastitis test, but also acute mastitis cases. Only data from Danish Holstein cows were considered in the analyses.

As a first step, data irrelevant for udder health management were removed: clinical registrations and treatment recordings in the database are related to various diseases, but only clinical registrations pertaining to the udder or the mammary gland, registered as the Danish equivalents of "udder" or "mammary gland," or results from the California mastitis test were kept. Treatments were considered relevant if they were registered as dry-cow treatment, pertaining to the udder or for diagnosed pathogens causing IMI.

As we were interested in treatment patterns in relation to udder health management, in the second step, we split the data set into 3 parts. The first part included 518 herds with available mastitis PCR results, clinical registrations, and treatment recordings in relation to udder health; the second part included 370 herds without PCR but with available clinical registrations and treatment recordings, and the third part consisted of 424 herds with only treatment recordings available.

From the milk recordings of these farms, average milk yields per parity were calculated for every cow and SCC values were log-transformed. Milk yields recorded as 0 or not available (**NA**), where SCC was also NA, were discarded because they were considered to be automated recordings for cows that were not actually milked (e.g., cows that were just dried off). Log-transformed SCC values that were given as negative infinity were regarded as NA because a SCC of 0 should not be possible. Parity and DIM were calculated according to the given calving dates. Parity was categorized as 1, 2, or $\geqslant 3$, and DIM were categorized as lactation stages in early (0–30 DIM), mid (31–250), late (251–450), and very late ($>$ 450 DIM) lactation. Observations in the last lactation of a cow were marked according to animal movements showing

death of the cow, with NA signaling that neither death nor a following lactation could be identified. Treatment registrations within 14 d of a previous registration were considered part of the same treatment (Barkema et al., 1998), except if a subsequent treatment was registered as dry-off treatment, which was always kept. The PCR was recorded for each tested pathogen (*Staphylococcus aureus*, other staphylococci including CNS, enterococci, *Corynebacterium bovis*, *Escherichia coli*, *Streptococcus dysgalactiae*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Klebsiella* spp., *Serratia marcescens*, *Arcanobacterium pyogenes* and *Peptostreptococcus indolicus*, *Mycoplasma bovis*, *Mycoplasma* spp., *Prototheca* spp., β-lactamase, yeast), but reduced to 1 observation in the data set with the minimum cycle threshold (**CT**) value. A PCR result with a CT value below 37 was considered positive, as this is the usual cut-off value used in Denmark for antibiotic treatments. Multiple clinical registrations made for 1 animal on the same day with the same result were considered as only 1 recording. As PCR recordings based on milk from the Danish milk recording scheme started in 2009, only data from 2009 onward were taken into account.

Finally, the 3 parts of the data set were transformed in 2 ways to account for possible differences between lactational and dry-off treatments. For lactational treatments, each recorded treatment led to one treatment observation and no-treatment observations were taken for each lactation stage (early, mid, late, very late) without a lactational treatment following in the same lactation/parity. For instance, a cow without treatment would lead to around 3 to 4 no-treatment observations per lactation (depending on when it was dried off), whereas a cow treated in mid lactation would have a treatment observation in mid lactation and may have a no-treatment observation later in lactation. Dry-off treatments were not considered in the data set for lactational treatments, though it was noted if a dry-off treatment occurred at the end of a lactation. For dry-off treatments, there was one observation per parity. In both cases, treatment and no-treatment observations were linked with the corresponding farm and cow, and to the following factors: lactation stage, parity, last average milk yield, and last log-transformed SCC before the observation, as well as information about prior PCR testing (positive PCR or

negative/no PCR), clinical registrations (yes/no), and whether or not the observation was in the cow's last parity (yes/no/NA). This is to account for whether the cow was culled or not. Observations in the dry-off treatment data set were additionally linked to information about lactational treatments in the same lactation (yes/no). Observations where parity was unknown were removed.

This led to 1 data set for lactational treatments and 1 for dry-off treatments, each with 3 parts (with PCR and clinical registrations, without PCR, without PCR or clinical registrations).

Statistical Analysis

For the statistical analyses, the data sets were subdivided into smaller data sets, each representing one farm and only including observations of that farm. All computations were done in the statistical computing software R version 3.3.1 "Bug in Your Hair" (R Core Team, 2016), using the additional packages data.table (Dowle et al., 2015), zoo (Zeileis and Grothendieck, 2005), lme4 (Bates et al., 2015), and ROCR (Sing et al., 2005). Figures were made using the packages ggplot2 (Wickham, 2009), ggbiplot (Vu, 2011), and dendextend (Galili, 2015).

LOGISTIC REGRESSION ANALYSIS. To investigate whether average milk yield, log-transformed SCC, PCR, and clinical registration can predict treatment, we performed farm-wise logistic regression. Adding parity, lactation stage, and information about whether or not the cow was in her last lactation as categorical covariates in a multivariable logistic regression to predict lactational treatments in a combined model leads to

$$\begin{aligned}\operatorname{logit}\left[P\left(\mathrm{TREAT}_i\right)\right] &= \beta_{i0} + \beta_{i1}\mathrm{AVGMY}_i + \beta_{i2}\log \mathrm{SCC}_i \\ &+ \beta_{i3}\mathrm{PCR}_i + \beta_{i4}\mathrm{CLIN}_i + \beta_{i5}\mathrm{PAR}_i + \beta_{i6}\mathrm{LAC}_i + \beta_{i7}\mathrm{LAST}_i \\ &+ \mathrm{COW}_i.\end{aligned}$$

As observations for dry-off treatments were always taken at the end of a parity, lactation stage was removed in this case, and instead information about whether there was a treatment dur-

ing the same lactation was added to predict dry-off treatments, leading to

$$\begin{aligned}\operatorname{logit}\left[P\left(DCT_i\right)\right] = \beta_{i0} + \beta_{i1}AVGMY_i + \beta_{i2}\log SCC_i \\ + \beta_{i3}PCR_i + \beta_{i4}CLIN_i + \beta_{i5}PAR_i + \beta_{i6}LTREAT_i \\ + \beta_{i7}LAST_i + COW_i.\end{aligned}$$

The left-hand sides of the equations, $P\left(TREAT_i\right)$ and $P\left(DCT_i\right)$, are the probabilities of lactational and dryoff treatment, respectively. AVGMY (last average milk yield), log SCC (last log-transformed SCC), PCR, CLIN (clinical registration), PAR (parity), LAC (lactation stage), LTREAT (treatment during lactation), and LAST (cow's last lactation) are the above-mentioned predictors, and $COW \sim N\left(0, \sigma_{cow}\right)$ is a random effect of cow. As our analyses were all farm-wise, $i = 1, \ldots 1312$ was a farm index. For farms without PCR or clinical registrations, the corresponding variables (PCR, CLIN, or both) were removed.

To evaluate how well the multivariable logistic regression models predicted treatment, models were also additionally fitted on subsets of data, where 10 % of the cows at each farm were randomly excluded when fitting the model and then used for testing model predictions. When predicting treatment for new cows, the model used the average population-level values for the random cow factor. The area under the receiver operating characteristic curve (**AUC**) was then calculated to evaluate the predictive capability of the models.

PRINCIPAL COMPONENT ANALYSIS. We used the coefficients of the variables obtained by logistic regression in principal component analyses (**PCA**) to investigate similarities or differences between the farms regarding treatment and treatment determinants. For numerical stabilization, farms with extreme coefficient values were excluded from the PCA. Because not all coefficients were significant, we decided to take the range of the significant coefficients of farms in the same data set and with the same type of registrations (available PCR recordings or clinical registrations) as a scale and considered values as extreme if they were outside of that range. Farms where the logistic regression did not converge were also excluded, leading to 422 farms (325

farms without PCR, 334 without PCR or clinical registrations) included in the PCA on lactational treatment coefficients and 381 farms (274 farms without PCR, 213 farms without PCR or clinical registrations) included in the PCA on dry-off treatment coefficients. The variables were centered (to 0) and scaled (to unit variance) before PCA was performed.

The rotated regression coefficients where clustered, using Ward's clustering criterion (Ward, 1963) with a cut-off value of 3 clusters. The number of clusters was chosen by a visual inspection of the corresponding dendrograms.

RESULTS

The number of observations per farm differed greatly among farms. In the data set for lactational treatments, numbers ranged from 639 (for farms with PCR recordings; 421 for farms with only clinical registrations; 129 for farms without clinical registrations or PCR) to 15,610 (11,980; 7,795) observations with 79 (34; 2) to 4,969 (3,053; 1,354) cases (treatments) and 424 (310; 70) to 13,090 (8,924; 7,732) controls (nontreatments) across a mean number of 42 (25; 9) to 1,317 (844; 547) cows per farm per year. The corresponding numbers for dry-off treatments were 285 (147; 64) to 10,640 (6,523; 3,775) observations with 1 to 2,348 (2,162; 903) cases and 249 (142; 53) to 9,487 (4,361; 3,770) controls. Fourteen (38; 91) farms had no registered dry-off treatments. Distributions of observations are given in Table I.1.

The multivariable logistic regression for lactational treatments, for herds with both PCR and clinical registrations, showed similar significance for all factors on the majority of the farms, though slightly less for parity (Figure I.1). The coefficients themselves showed a higher probability of treatment with a higher SCC, higher milk yield or later in lactation (Figure I.2). Clinical registration coefficients suggested higher probability for treatment with a clinical registration, whereas PCR coefficients indicated a lower probability for treatment with a positive PCR. Similarly, cows in their last lactation (before culling) mostly had a lower probability for treatment (Figure I.2). Parity coefficients were more centered around 0 with a small shift to the left, indicating

Item	Treatment observations					Nontreatment observations				
	Minimum	1st quarter	Median	3rd quarter	Maximum	Minimum	1st quarter	Median	3rd quarter	Maximum
Lactational treatment										
PCR	0	1	6	15	306	0	12	128.5	318	3,193
Clinical registrations	0	113.8	269	501	3,713	0	704.8	1,346	2,043	7,618
Average milk yield	24.05	32.32	34.15	36.23	46.71	21.38	30.14	31.72	33.31	42.54
Average SCC	122.7	558.7	715.8	912.4	2,057	137.2	269	330.8	395.6	773.9
Cows in their last lactation	7	116.2	173	258.8	1,393	6	617	783	1,046	3.270
Cows in parity 1	17	110	172.5	270.2	1,839	93	900.8	1154	1,632	5,580
Cows in parity 2	20	120.2	183	278.8	1,571	67	626.5	815	1,130	3,804
Cows in parity $\geqslant 3$	32	177	271.5	417.8	1,750	66	685.2	931	1,279	4,011
Cows in early lactation	18	105	159	248	1,407	20	576.5	777	1,099	7,652
Cows in mid lactation	26	148	227	356	2,156	131	871.8	1,156	1,611	4,915
Cows in late lactation	3	106	194.5	332.8	2,608	73	687	888	1,221	3,894
Cows in very late lactation	0	7	14	25	271	2	57.25	81	119.8	474
Mean number of cows	9.38	42.41	64.31	90.91	432.4	33.38	156.8	204.4	281.3	1,235
Dry-off treatment										
PCR	0	2	56	174	1,138	0	7	45	132.2	1,013
Clinical registrations	0	71	213.5	389.2	2,282	0	366.8	667	1,046	3,446
Average milk yield	22.54	29.97	32.1	33.91	43.5	20.33	29.4	31.03	32.97	42.2
Average SCC	112.4	290.8	379.8	484.6	2,602	167.7	343.6	429.4	515.4	1,059
Cows in their last lactation	0	2	5	10	115	4	313.2	409	527	1,740
Cows in parity 1	0	44	110	210.2	1,015	75	333.5	458	609.5	3,829
Cows in parity 2	0	46	91	164	732	53	249.2	331	443.5	2,643
Cows in parity $\geqslant 3$	0	47.75	93.5	168.5	756	55	311.2	417.5	584.8	3,015
Cow with earlier treatment	0	70	153.5	294.5	2,121	15	209.2	288	417.5	1,401
Mean number of cows	1	21.64	41.05	70.47	354.6	30.12	108.7	145.2	195.5	1,137

Table I.1: Summary statistics for number of positive PCR and clinical registrations, average milk yields (in kg), average SCC (in thousands), total number of cows in parity and lactation groups, and mean number of cows per farm between 2009 and February 2016, split between treatment and nontreatment observations for lactational and dry-off treatments on all farms with PCR and clinical registrations

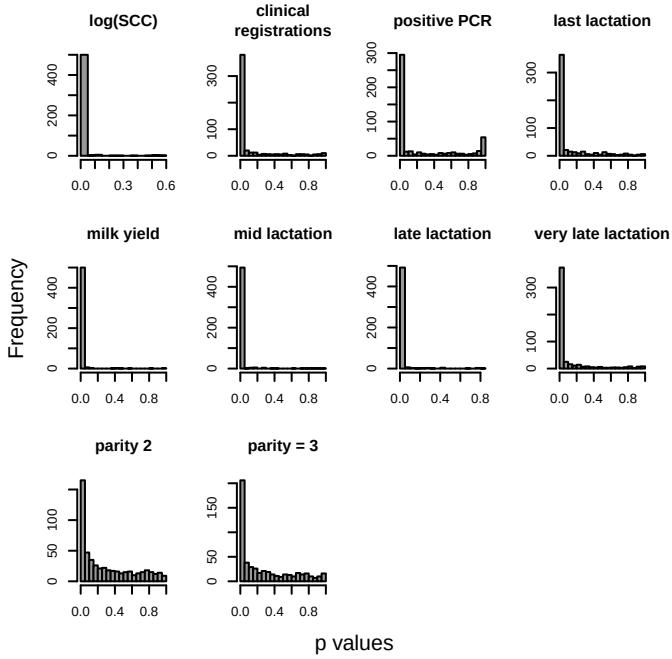

Figure I.1: Histogram of P-values in multivariable logistic regressions of lactational treatments for farms with PCR and clinical registrations.

slightly lower treatment probability for higher parities. This was especially observable in the significant coefficients (Figure I.3). For most farms, only some of the coefficients were significant, but for 124 farms (39 farms with PCR and clinical registrations, 47 farms without PCR, 38 farms without PCR or clinical registrations), all coefficients were significant.

Multivariable logistic regression results for dry-off treatments for herds with PCR and clinical registrations were comparable to those for lactational treatments (Figures I.4 and I.5), though there were more extreme values in the regression coefficients (results not shown). Notable differences could be seen for PCR, where coefficients suggested a higher probability for dry-off treatment given a positive PCR result. A higher probability for treatment was also indicated by coefficients for preceding lactational treatments (Figure I.5). On 86 farms (37 farms including PCR, 27 farms without PCR, 22 farms without PCR or clinical registrations), all coefficients were significant.

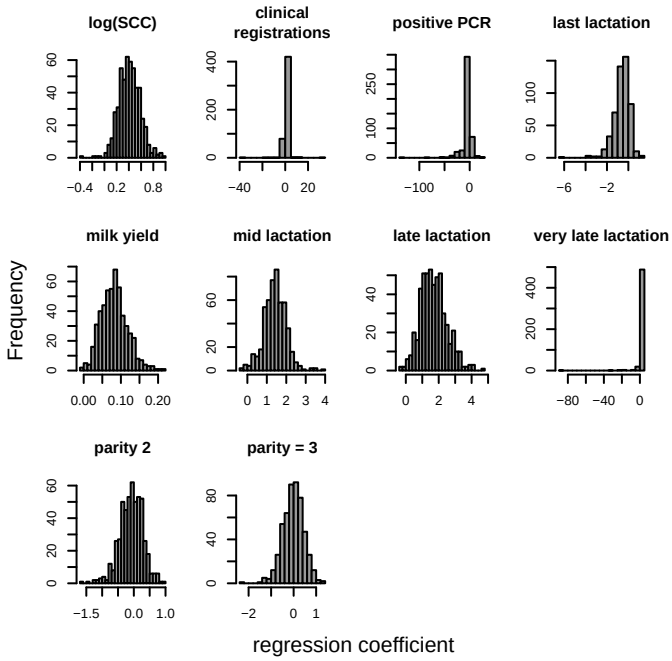

Figure I.2: Histogram of coefficients in multivariable logistic regressions of lactational treatments for farms with PCR and clinical registrations. Farms with extreme coefficients (see Figure I.3) are removed from further analysis.

Model validation by fitting the regressions on 90 % of the cows in each model showed very good model fit for the remaining 10 % with the mean AUC at 76 % for lactational treatments and 85 % for dry-off treatments, and the median AUC at 76.3 % (lactational) and 85.8 % (dry cow, Figure I.6).

The PCA results showed that for both types of treatments the first 2 principal components explain more than 50 % of the variance (Figures I.7 and I.8). These 2 components included all used predictors, although to varying degrees.

Clustering by Ward's clustering criterion on all principal components for lactational treatments showed 3 clusters (Figure I.9), one of which was aligned with parity, clinical registrations, and PCR. The second cluster was aligned around average milk yield and lactation stage, and the third cluster was aligned around a cow's last lactation. The SCC seemed to be between this last and the first cluster (Figure I.7). For dry-off treatments, farms

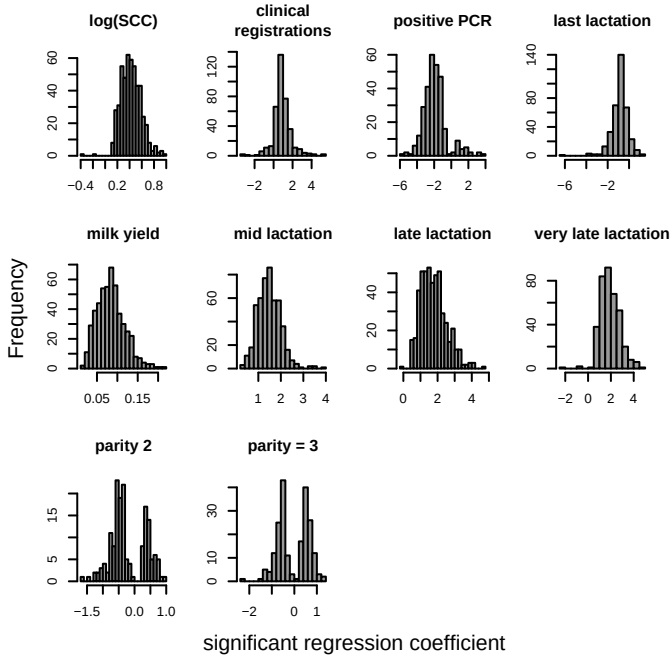

Figure I.3: Histogram of significant coefficients in multivariable logistic regressions of lactational treatments for farms with PCR and clinical registrations. The depicted ranges were taken as standard for the coefficient ranges in Figure I.2.

seemed to cluster mainly around average milk yield and between parity and a cow's last lactation, with a smaller third cluster aligned with clinical registrations, PCR, SCC, and lactational treatments in the same lactation (Figures I.8 and I.10). For farms without PCR, clustering for dry-off treatments added several small clusters before more than one big cluster appeared (results not shown).

The included figures show results for farms with PCR and clinical registrations. Specific results of the logistic regression, clustering and PCA for herds without PCR, and herds without PCR or clinical registrations are not shown nor further discussed separately, as they displayed similar trends for both lactational and dry-off treatments.

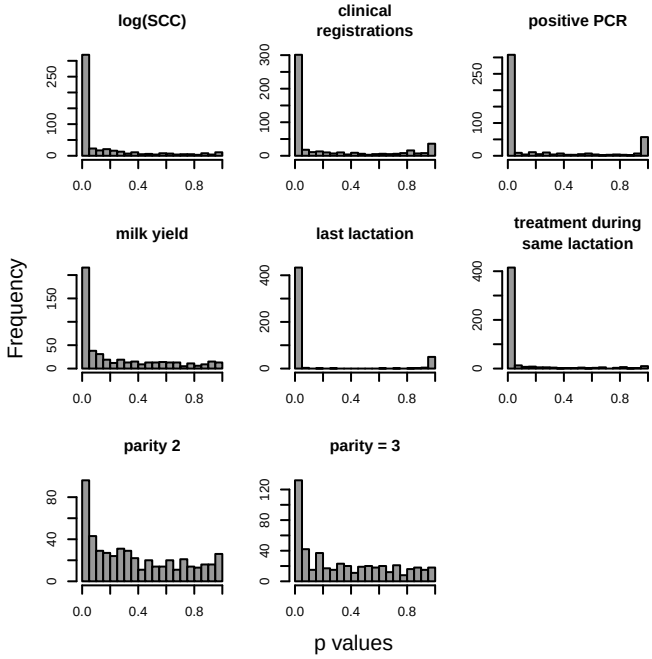

Figure I.4: Histogram of P-values in multivariable logistic regressions of dry-off treatments for farms with PCR and clinical registrations.

DISCUSSION

Milk yield recording data, including SCC, are collected regularly on most farms for all cows, leading to near-complete information, whereas PCR has to be transferred and clinical registrations have to be entered into the database manually by the veterinarian or the farmer, who might forget to register this information, sometimes leading to incomplete data or registration errors. Nevertheless, Wolff et al. (2012) investigated the completeness and quality of the national database registers in Denmark, Finland, Norway, and Sweden. The authors found that the Danish register regarding clinical mastitis had the highest quality and around 90 % completeness, which increases our confidence in the outcomes of the current study. Still, registration errors do occur, and during data management, we encountered some of those such as recordings of dry-off treatments during early lactation, which are most likely treatments for clinical mastitis.

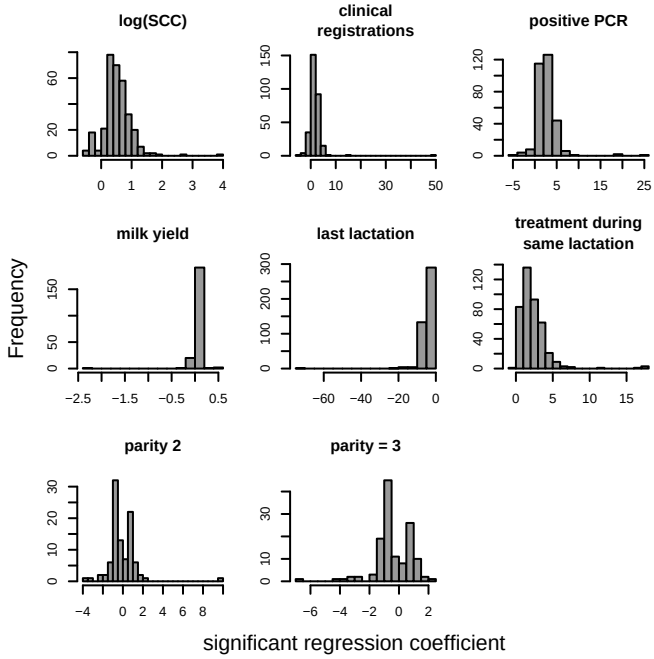

Figure I.5: Histogram of significant coefficients in multivariable logistic regressions of dry-off treatments for farms with PCR and clinical registrations. Values outside of the depicted ranges were removed as extreme values.

In this study, we conducted untraditional farm-wise logistic regressions because we were interested in both individual farms and in differences between the farms, and not in generic or average estimates corrected for the farm effect. By estimating logistic regression parameters for each farm, we obtained information about individual farms (farm-specific), which could then be used to investigate differences between the farms. We also distinguished between lactational and dry-off treatments, where those were recorded.

Our logistic regression analyses showed that, on many farms, a high SCC and high milk yield are associated with a higher probability of treatment, both for lactational and for dry-off treatments, as is a clinical registration. Cows that were treated during lactation also had a higher probability for dry-off treatment. It could be that farmers treat cows at dry-off that had a treatment (a mastitis problem) during the lactation, whether they need

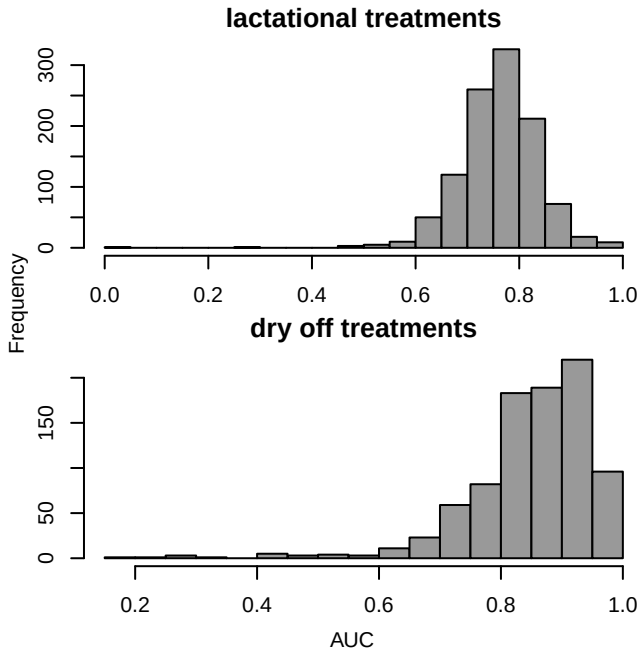

Figure I.6: Histogram of area under the receiver operating characteristics curve (AUC) for all farms without extreme coefficients for (a) lactational treatments and (b) dry-off treatments. Model performance was tested on 10 % of the cows, which were not included in model fitting.

or do not need the treatment. Earlier studies (e.g., Steeneveld et al., 2008; Waage et al., 1998; Zadoks et al., 2001) have on the other hand shown that high milk production and SCC, as well as previous IMI, are risk factors for clinical mastitis that may lead to antibiotic treatment. These factors may therefore just indicate that there was an IMI (likely to be chronic) that had to be treated. However, it is also expected that farmers would like to keep cows that are performing better than the average cow; therefore, they may rather treat such cows in case of a potential udder health complication to ensure optimal performance of the cows (according to the farmer's belief). Also, because blanket dry cow therapy is not allowed in Denmark, SCC is one of the main indicators used for selecting cows for testing (using bacterial culture or PCR), subsequently allowing dry-off treatment with antibiotics if the cow is tested positive. If, on top of that, farmers decide to

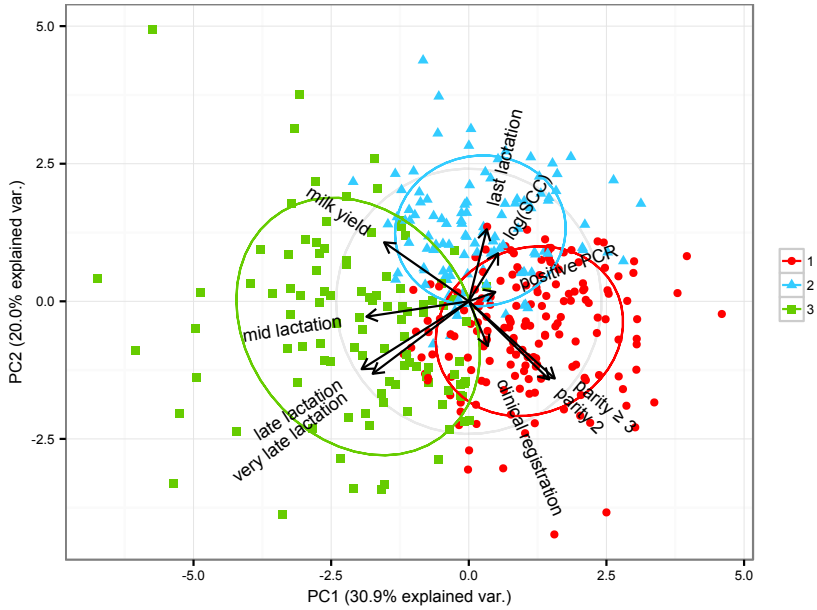

Figure I.7: Principal component analysis (PCA) biplot with Ward's clustering criterion for lactational treatments on farms with PCR and clinical registrations. PC1 = principal component 1; PC2 = principal component 2; var. = variance. Color version available online.

select cows for testing based on whether or not they already had mastitis and mastitis treatment, the associations between dry-off treatments and SCC or preceding lactational treatments could be further explained.

A positive PCR was also associated with a higher probability of dry-off treatment, which can be explained by the fact that Danish legislation allows farmers to use dry cow therapy on PCR-positive cows (Figure I.5). On the other hand, a positive PCR seemed to lower the probability for a treatment during lactation (Figures I.2 and I.3). To find a satisfactory explanation for this, a more thorough understanding of when and why farmers decide to use PCR, specifically in relation to lactational treatments, is needed.

For both types of treatment, the regression coefficients showed that cows were rarely treated in their last lactation. This is likely explained by the farmer choosing to cull instead of treating a cow.

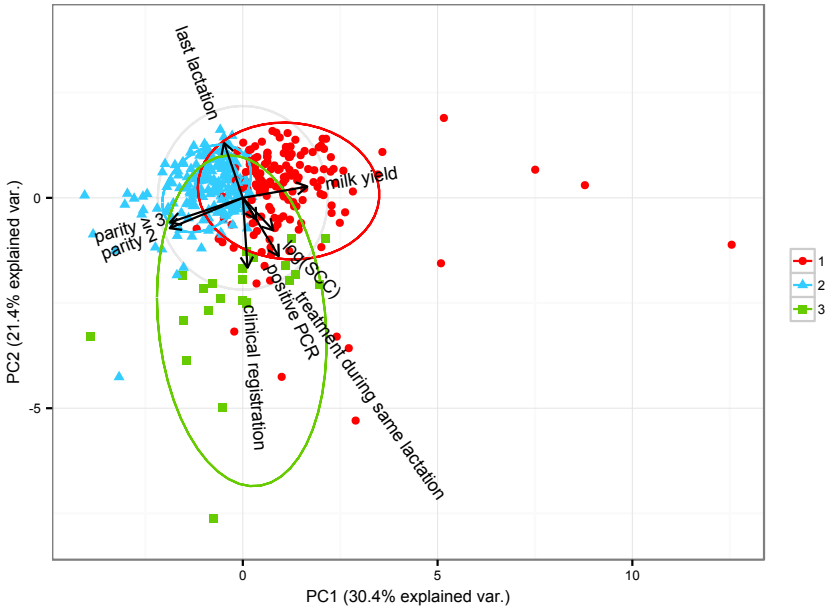

Figure I.8: Principal component analysis (PCA) biplot with Ward's clustering criterion for dry-off treatments on farms with PCR and clinical registrations. One observation (principal component 1 (PC1) = 2.8 and principal component 2 (PC2) = −13.2) in group 3 is not shown in the plot. Var. = variance. Color version available online.

The multivariable logistic regression showed different farmer behavior toward treatment when it comes to the parity of the cows (Figures I.2, I.3, and I.5). There seem to be farmers that tend to treat younger cows rather than older ones, as well as farmers that treat the older ones rather than the younger cows. As younger cows are considered the future potential of the farm, the decision to treat instead of cull in case of an udder health complication may not be surprising. On the other hand, a farmer may decide to keep only higher producing cows and treat those, even if they are older, or hope that younger cows can clear an infection more easily without treatment.

Our results from the PCA and clustering indicated 3 big clusters for lactational treatments (Figure I.9) and 2 big and 1 smaller cluster for dry-off treatments (Figure I.10). In both cases, 1 cluster covered farms where farmers mostly concentrated on health indicators such as positive PCR, clinical registrations, and SCC.

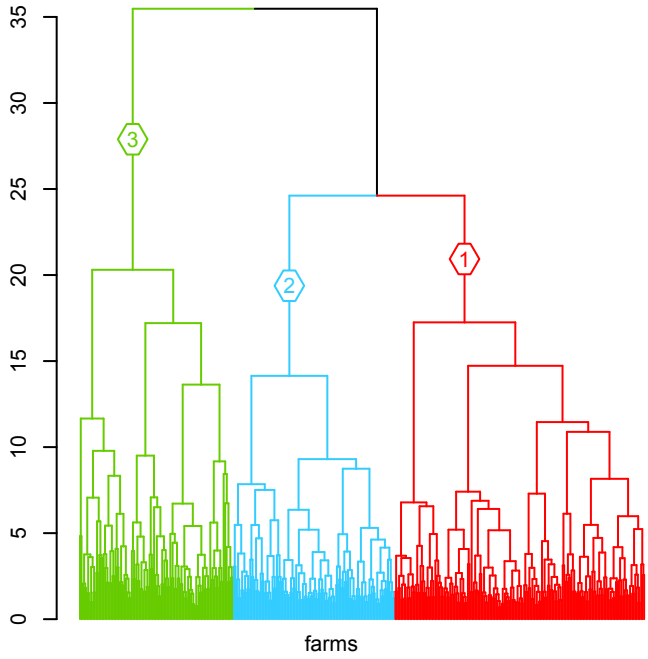

Figure I.9: Dendrogram of Ward's clustering criterion (lactational treatments on farms with PCR and clinical registrations). The clustering height (y-axis) is given in variance units according to Ward's clustering criterion. Color version available online.

For dry-off treatments, treatments in the same lactation were included in these health indicators, and this cluster was the smallest. Another cluster covered farmers whose decision to treat was based mostly on production factors like average milk yield, and in the case of lactational treatments, DIM, keeping the "more profitable" (high producing) cows. The third cluster for lactational treatments seemed to be centered around a cow's last lactation, also partly including SCC. For dry-off treatments, the third cluster seemed mostly influenced by parity and a cow's last lactation. This may indicate that the farmers' decision to cull instead of treating cows during the lactation is affected by the SCC of the cow, whereas the decision for dry-off treatments may be more affected by the age of the cow.

The decision for treatment may also be influenced by the consulting veterinarian. As we do not account for the veterinarian in

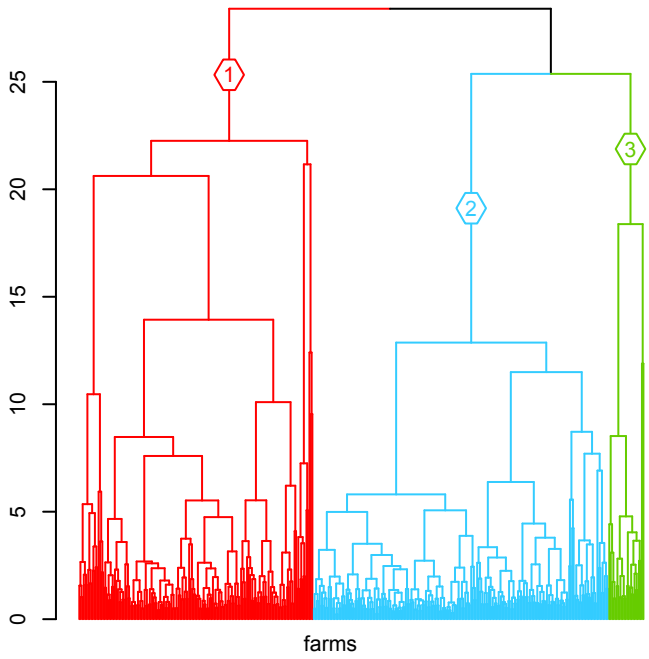

Figure I.10: Dendrogram of Ward's clustering criterion (dry-off treatments on farms with PCR and clinical registrations). The clustering height (y-axis) is given in variance units according to Ward's clustering criterion. Color version available online.

our analyses, it is possible that the clustering may be influenced by the herd veterinarian. Further studies could investigate the veterinarian's influence by including the veterinarian as an effect in the model. The farmer himself may also have biased our results by his perception of which cases should receive treatment, because some farmers may add to the clinical registrations. Still, we expect this bias to be minor because the majority of the cases are registered by the veterinarian.

Cows with clinical mastitis that were not treated but were for instance culled or slaughtered were not considered separately, as culling determinants on different farms were out of the scope of this study. Nevertheless, we tried to adjust for culling by including a binary variable indicating if a cow was in the last lactation. As expected, cows in their last lactation were rarely treated. Further studies focusing on understanding determinants

for culling of dairy cows or the more specific relation between treatments and culling may use the methods presented in this study.

We chose a cut-off at a CT value of 37 to define a positive or negative PCR result and we did not consider other cut-off values, nor the pathogen that the PCR reacted to. The cut-off value of 37 is the value that permits treating cows with antibiotics in Denmark, which farmers generally use. Farmers are allowed to treat cows with antibiotics based purely on a positive PCR result, regardless of the pathogen the PCR reacted to. If they only test cows that they think should be treated, any positive PCR might result in treatment.

Our results clearly show that farmers behave differently. For instance, the results show that health indicators are most indicative for some farms, whereas others use production-related factors (Figures I.7 and I.8). In addition, variations in the extent of the determinants' effects are clear between farms (Figures I.2, I.3, and I.5). This indicates that a herd-specific approach for udder health improvement with a focus on optimizing the use of antibiotics may be useful. Simulation models could be used to examine and gradually adjust farm-specific udder health management programs under different circumstances (e.g., the level of the mastitis problem in the herd, the causative agent of mastitis, the farmer's way of selecting cows for treatments with antimicrobials, and with different assumptions about the current treatment regimen). This will allow for cost-effective changes of control programs, without having to adopt a totally different strategy. Thereafter, the information can be communicated by the veterinarian or the milk quality advisors (or both) to provide farm-specific advice not only based on the farmer's statement about his udder health management, but also augmented with available data. Herd-specific control programs that consider a farmer's behavior toward selection of cows for antimicrobial treatments may motivate the farmer to adopt new mastitis control programs resulting in not only improving udder health cost-effectively, but also enhancing prudent use of antibiotics.

CONCLUSIONS

Danish cattle database recordings can be used to find determinants for antibiotic treatment in relation to udder health. Determinants that were most important for predicting antibiotic treatments vary from one farm to another. Health indicators such as PCR or SCC were most indicative for treatment on some farms, whereas other groups seemed to depend more on production factors (milk yield) or later culling of the cows. This shows that farmers behave differently and differences can be identified in register data. Hence, a data-assisted farm-specific approach to improve udder health, which considers how the farmer selects animals for antibiotic treatments, may prove useful in motivating the farmer to adopt the proposed approach. This would improve udder health and encourage prudent use of antibiotics.

ACKNOWLEDGEMENTS

This study is part of the STOPMAST project, funded by the Danish Milk Levy Foundation (Mælkeafgiftsfonden). The authors thank Jørgen Nielsen and SEGES (Aarhus, Denmark) for providing the data.

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4 MODELLING IMI AND INTERVENTIONS

The main part of the PhD project was related to simulation modelling, or more specifically, modelling prevention and control of mastitis in dairy herds. Modelling different intervention strategies against IMI allows their comparison regarding cost-effectiveness, and is thus an important step in improving mastitis control.

However, as described in Section 2.2.2, already existing models were missing certain factors. Therefore, a new model addressing these gaps and fulfilling all requirements (see Section 2.2.2 and Figure 3) was needed. In short, the model should simulate a dairy herd with milk production, pathogen transmission, and intervention measures. The here proposed model uses a recently described dairy herd model (Kirkeby et al., 2016), with a newly added transmission framework for IMI (Manuscript II), and intervention measures against clinical and subclinical IMI (Manuscripts III and IV).

In this chapter, the model and intervention strategies are presented in Sections 4.3, 4.4, and 4.5, after a short summary of the model, highlighting the requirements mentioned in Section 2.2.2. Furthermore, additional one pathogen scenarios not presented in Manuscript II are shown in Section 4.2.

4.1 MATERIALS AND METHODS

In the following, a short description of the model is given. A more thorough description of the transmission framework, IMI effects on production, and voluntary culling can be found in Section 4.3. Selected interventions against clinical and subclinical IMI are described and investigated in Sections 4.4 and 4.5, respectively.

4.1.1 Herd model

The base herd model was taken from the iCull model (Kirkeby et al., 2016), that was originally used to investigate paratuberculosis control actions.

In the model, the herd is initiated by drawing a number of animals in every category (calves, heifers, inseminated heifers, pregnant heifers, lactating cows, inseminated lactating cows, pregnant lactating cows, and dry cows), depending on a target count (default value 200 cows) and a demographic distribution. Then, a closed dairy cattle herd is modelled with daily time steps. Upon entering a category, the number of days the animal will stay there is drawn from a distribution, before it moves on to the next category, again. As an example, a pregnant lactating cow could be dried off, stay a dry cow for 56 days, calve, and return to being a lactating cow for a number of days.

The base iCull also models daily milk yield (originally energy corrected milk) and SCC for every lactating cow, including protein and fat percentages, which are used to calculate the income from milk. Milk yield recordings are simulated once a month. Feeding costs for lactating cows depend on the modelled milk yield, for other animals, feeding costs are fixed.

Culling is divided into involuntary and voluntary culling. Once a week, animals are evaluated for voluntary culling, if the target count (see above) is exceeded. Milk production, reproduction status, SCC, and parity are weighted and cows with the highest weight are culled.

Furthermore, the base model includes the option to estimate the future average milk production (FAP) of a cow, depending on, among other things, its milk yield and recorded SCC (Græsbøll et al., 2017).

4.1.2 IMI transmission framework

IMI transmission is similar to the bovine IMI model (Halasa et al., 2009b), but modelled at quarter level. Furthermore, infection (risk factors, Zadoks et al., 2001a) and cure after treatment (Steeneveld et al., 2011) are cow-specific. To demonstrate differences between strains, two *S. uberis* strains are included,

together with three other pathogens (*S. aureus*, *S. agalactiae*, and *E. coli*). Each pathogen strain has an assigned transmission mode, three of which are available (contagious, environmental, and opportunistic—a mixture of contagious and environmental, see Section 2.1.1 and Figure 2). The model is therefore strain-specific.

For dry cows, IMI transmission is independent of the number of infected quarters, and clinical cases can only occur in the first and last week of the dry period (Halasa et al., 2010). For cows that received DCT, transmission rates are lower, and quarters with subclinical IMI cannot flare up.

Transmission rates in general can be easily adjusted to account for different herd situations.

4.1.3 Effects on production

The effects of IMI on milk production are modelled for both clinical and subclinical IMI. For subclinical IMI, the SCC is increased, depending on the causative pathogen (Schepers et al., 1997; Wilson et al., 1997); and milk yield is lowered (Hortet et al., 1999). For clinical IMI, milk yield is reduced until the end of lactation, also depending on the pathogen (Gröhn et al., 2004). This part was coded by Carsten Kirkeby.

4.1.4 Interventions

Voluntary Culling

As described above, SCC, which can be seen as an indicator for subclinical IMI (see Section 2.1.1), was already considered in voluntary culling decisions. In the MiCull model, previous cases of clinical IMI were added as an additional weight for voluntary culling (Bar et al., 2008a).

Dry Cow Treatment

If a cow gets a clinical IMI in the first week of the dry period, it will be treated with DCT. On top of that, cows are selected for testing, if they had a clinical IMI during the lactation, or if they

had a high SCC at a milk recording within the last three months. Testing is done by PCR, and if the test returns a positive result, DCT will be administered.

Clinical Interventions

The default intervention against clinical IMI is a three day intramammary treatment of all clinical cases. Other intervention options include longer treatment or reactive culling. Not recovered cows can be culled after treatment or cows can be culled instead of treated, for example if they had repeated cases of clinical IMI or after diagnostic testing of clinical quarters to determine the causative pathogen. Based on the test result, an expected recovery probability is estimated and cows below a certain threshold (0.5 or 0.75) are culled instead of treated. The culling threshold can be adjusted for primiparous cows. Clinical intervention strategies are investigated in Manuscript III (Section 4.4).

Subclinical Interventions

By default, there are no intervention measures against subclinical IMI. In all options for subclinical interventions, cows with two subsequent high SCC are tested for IMI and test positive cows are treated. The test can be repeated a month later, and cows that are still tested positive are culled. There are also cow-specific intervention options. Interventions against subclinical IMI in combination with different clinical intervention measures are investigated in Manuscript IV (Section 4.5).

Furthermore, for clinical IMI that persisted as subclinical IMI after treatment, the cure probability after treatment for subclinical IMI is reduced to the minimum of the cure probabilities for treatment of clinical and subclinical IMI. This option can be used or ignored in the model.

4.2 RESULTS NOT PRESENTED IN THE PAPERS

In addition to the one pathogen scenarios presented in Manuscript II, eight one pathogen scenarios (for *S. aureus*, *S. agalactiae*,

contagious *S. uberis*, or environmental *S. uberis*) are presented in Table 1 and Figure 4. The results show that the per case costs, particularly for subclinical cases, increased with decreasing transmission rate, i.e. with decreasing number of cases.

This phenomenon can be explained by culling dynamics and costs caused by milk loss. In a closed herd, the farmer can only cull a certain number of animals, as replacement heifers have to be available. In general culling decisions, involuntary culling and culling for acute IMI have the highest priorities. Clinical cases have a fixed low probability to become acute, so higher prevalences will also lead to more acute cases. Consequently, less cows with subclinical IMI will be culled with a high SCC. These animals will stay in the herd and the additional lost milk increases the per case costs.

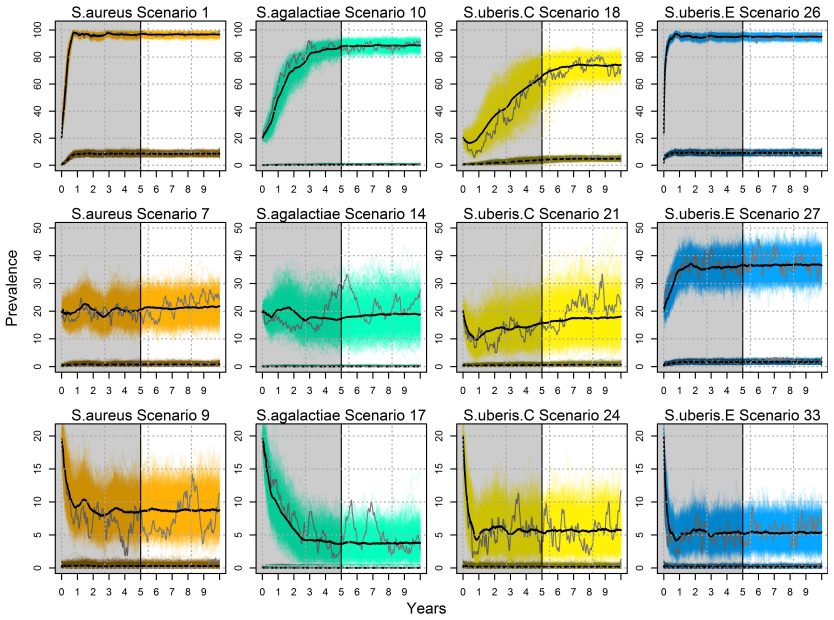

Figure 4: Daily cow level prevalences in the one pathogen scenarios described in Table 1. Every scenario shows 500 iterations over five years with an additional five year burn-in period. One random iteration is shown in gray, the mean is displayed in black. The top row shows scenarios with unadjusted transmission rates. Scenarios 7, 9, 14, and 21 are also presented in Manuscript II.

Scenario	Pathogen	Transmission rate scaling factor	Transmission rate	Transmission rate (dry cows)	culled with high SCC	Per case cost, subclinical	Per case cost, clinical
1	*S. aureus*	1	0.0179	0.0179	60 (52, 67)	28 (27, 29)	337 (320, 354)
7	*S. aureus*	0.2	0.0036	0.0036	22 (18, 26)	86 (78, 94)	267 (197, 356)
9	*S. aureus*	0.1	0.0018	0.0018	13 (10, 16)	100 (86, 114)	226 (173, 315)
10	*S. agalactiae*	1	0.0068	0.0011	66 (59, 73)	118 (111, 125)	95 (80, 117)
14	*S. agalactiae*	0.55	0.0037	0.0006	24 (19, 29)	369 (309, 442)	121 (88, 185)
17	*S. agalactiae*	0.25	0.0017	0.0003	10 (8, 13)	569 (462, 711)	154 (76, 348)
18	*S. uberis* (c)	1	0.0155	0.0011	51 (45, 56)	37 (34, 41)	114 (86, 156)
21	*S. uberis* (c)	0.8	0.0124	0.0009	15 (10, 20)	80 (69, 91)	175 (116, 281)
24	*S. uberis* (c)	0.5	0.0078	0.0006	6 (4, 9)	106 (89, 127)	191 (144, 259)
26	*S. uberis* (e)	1	0.0155	0.0011	58 (52, 66)	28 (27, 29)	267 (259, 276)
27	*S. uberis* (e)	0.1	0.0016	0.0001	31 (27, 35)	67 (63, 71)	306 (274, 344)
33	*S. uberis* (e)	0.01	0.0002	0.000 01	6 (4, 9)	109 (92, 130)	172 (144, 211)

Table 1: Median per case costs (with 5% and 95% percentiles) for subclinical and clinical IMI in € per year (mean over 5 years) in one pathogen scenarios. Scenarios marked in gray are also presented in Manuscript II. Numbers are rounded.

4.3 MANUSCRIPT II

A STRAIN-, COW-, AND HERD-SPECIFIC BIO-ECONOMIC SIMULATION MODEL OF INTRAMAMMARY INFECTIONS IN DAIRY CATTLE HERDS

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Published in *Journal of Theoretical Biology* 449:83–93
https://dx.doi.org/10.1016/j.jtbi.2018.04.022

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ABSTRACT

Intramammary infections (IMI) in dairy cattle lead to economic losses for farmers, both through reduced milk production and disease control measures. We present the first strain-, cow- and herd-specific bio-economic simulation model of intramammary infections in a dairy cattle herd. The model can be used to investigate the cost-effectiveness of different prevention and control strategies against IMI. The objective of this study was to describe a transmission framework, which simulates spread of IMI causing pathogens through different transmission modes. These include the traditional contagious and environmental spread and a new opportunistic transmission mode. In addition, the within-herd transmission dynamics of IMI causing pathogens were studied. Sensitivity analysis was conducted to investigate the influence of input parameters on model predictions. The results show that the model is able to represent various within-herd levels of IMI prevalence, depending on the simulated pathogens and their parameter settings. The parameters can be adjusted to include different combinations of IMI causing pathogens at different prevalence levels, representing herd-specific situations. The model is most sensitive to varying the transmission rate parameters and the strain-specific recovery rates from IMI. It can be used for investigating both short term operational and long term strategic decisions for the prevention and control of IMI in dairy cattle herds.

KEY WORDS: mastitis, mathematical model, cow-specific, pathogen-specific

II.1 INTRODUCTION

Mastitis or intramammary infection (IMI) is one of the most frequent and costly diseases in dairy herds, where costs arise from both milk loss and control measures (Halasa et al., 2007; Seegers et al., 2003). They can be caused by many different pathogens, traditionally differentiated into environmental and contagious. Contagious pathogens are thought to be transmitted during the

milking process (Harmon, 1994). They can cause outbreaks, infecting many animals in a short period of time resulting in high incidence rates (Zadoks et al., 2001a). In contrast, environmental pathogens are considered to be transmitted, among other things, through reservoirs in the stable and to have an endemic nature, associated with low incidence rates (Blowey and Edmondson, 2010; Zadoks et al., 2001a).

Traditionally, *Staphylococcus aureus* and *Streptococcus agalactiae* are examples for contagious pathogens, while *Escherichia coli* and *Streptococcus uberis* are considered as environmental. However, Zadoks et al. (2001a) described how *S. uberis* caused an outbreak like situation, suggesting that this particular pathogen strain was transmitted contagiously, indicating that differentstrains can have different properties. Consequently, control strategies should take the differences (in spread and recovery following treatment) between strains of the same pathogen species into account to be effective. Moreover, some pathogen strains may create reservoirs in the environment and yet express contagious transmission between cows, reflecting an "opportunistic" behavior that combines both contagious and environmental characteristics (Jørgensen et al., 2016). In order to capture this more differentiated behavior, we introduce a new transmission mode with both contagious and environmental characteristics at the same time, in contrast to a purely contagious or purely environmental transmission. In the model *S. agalactiae* is used as an example for the opportunistic nature of IMI causing pathogens.

Simulation models of IMI have previously been used to investigate the impact of different management strategies against IMI (e.g. Allore et al., 1998; van den Borne et al., 2010a; Hagnestam-Nielsen and Østergaard, 2009; Halasa et al., 2010; Østergaard et al., 2005; Steeneveld et al., 2011). Some of these models have been pathogen-specific, taking traditional transmission modes between pathogens into account (Halasa et al., 2009a, 2010). Others were cow-specific, taking risk factors for infection into account (Allore et al., 1998), or focusing on characteristics of the single cow (Steeneveld et al., 2011); or herd-specific, looking into differences between herds such as herds having different pathogens (Østergaard et al., 2005). However, to our knowledge, no previous models have been simultaneously strain-, cow- and herd-specific.

The model we propose considers the spread dynamics not only on species level, but also specifically distinguishes between different strains of the same species, for instance allowing future economic assessment of strain-specific diagnostics, perhaps on farm level. It includes the characteristics of the single cow for infection, recovery following potential treatment, and its future production potential, allowing a comparison of a cow to its herd mates, while modelling IMI transmission on quarter level. This allows investigating cost-effectiveness of control actions on quarter level, such as blinding (drying off) chronically infected quarters. In addition, the model includes differences between herds such as size, production and management. It is thus strain-, cow- and herd-specific and can be used as a tool to examine both short and long term decisions to prevent and control IMI for individual cows in individual herds, which is to our knowledge not available in previous bio-economic models.

The objective of this study is to describe a new transmission framework of IMI causing pathogens, including a new opportunistic transmission mode.

II.2 MATERIALS AND METHODS

II.2.1 Model framework

This study was conducted with the MiCull model (Mastitis-iCull), version 1.0. The model framework was created by combining an extension of the transmission framework for IMI created by Halasa et al. (2009a) with the iCull simulation model of a dairy herd described by Kirkeby et al. (2016), using R version 3.2.2 – "Fire Safety" (R Core Team, 2016).

The base iCull model is a stochastic mechanistic bio-economic model that simulates a dairy herd using single-day time steps to allow for both operational and strategic decision making (Kirkeby et al., 2016). In brief, the model simulates a dairy cattle herd in five different physical herd compartments: calves, heifers, lactating cows, dry cows and calving area. Each cow spends a random (drawn from a given distribution) predefined number of days in each compartment before moving on to the next, with

the exception of possible removal from the herd by culling or slaughter decisions (see II.2.3). Lactation curves and somatic cell count (SCC) curves are modelled for every cow and depend on cow-specific parameters, indicating the individual level relative to the mean in the herd (Græsbøll et al., 2016). Feeding is dependent on the life stage of each animal, and for lactating cows it is modelled based on the amount of milk produced (Kirkeby et al., 2016).

In the present MiCull model, lactation and SCC curves, as well as farmer decisions, were adjusted to include IMI related factors as described below. The transmission framework for IMI includes environmental (constant infection probability), contagious (infection probability depends on the number of infected quarters), and opportunistic (infection probability depends on the number of infected quarters or the presence of bacteria in the environment) transmission on quarter level, cow-specific infection and recovery, and different strains of the same pathogen.

II.2.2 Pathogen transmission

Pathogen transmission occurs on quarter level, i.e. between the quarters of dairy cows in a herd, independent of whether two quarters belong to the same or different cows. Each quarter can be in one of three infection states, susceptible (S), subclinically infected (I_s), and clinically infected (I_c), or it can be blinded (NA). At the moment, the model includes 5 different pathogen strains for demonstration purposes (Table II.1). Other pathogens or updated pathogen parameters can be easily added. New infections with IMI causing pathogens are handled differently for milking cows, dry cows and heifers as described below.

Susceptibility

The MiCull model is cow-specific, including in the infection process in lactating cows. Risk factors, such as parity and previous cases of clinical IMI (quarter-level), are used to adjust the susceptibility for IMI of a cow (Zadoks et al., 2001b). Cows in their first parity and quarters without prior IMI are taken as reference, and the risk ratios for cows in the second and third or

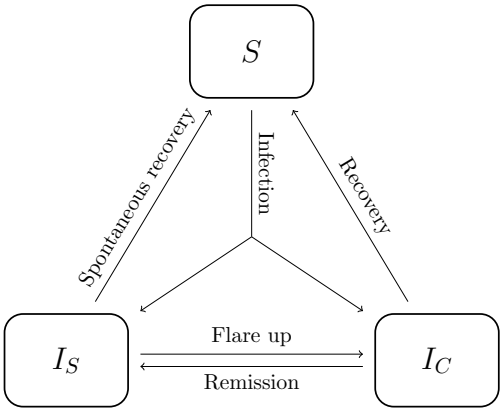

Figure II.1: Diagram of the transmission framework used in the simulation model.

higher parity ($RR^{(parity)}$), as well as for quarters with prior infections ($RR^{(prior)}$) are calculated (mean of *S. aureus* and *S. uberis*, Zadoks et al., 2001b). The product of all risk ratios pertaining to a quarter q is then used to adjust the susceptibility $Susc_q$ of the quarter for new IMI, $Susc_q = RR_q^{(parity)} \cdot RR_q^{(prior)}$. In equation (1), the susceptibility leads to an adjustment of the transmission rate, depending on cow parameters, thus leading to cow-specific infection.

Lactating cows

For lactating cows, all transitions between the three infection states are modelled (Figure II.1). The probability of infection for each quarter includes the susceptibility and is thus cow-specific.

In the model, all pathogen strains are identified by a unique strain ID (strain), and active pathogens are marked as such. Infections can occur for all active strains, and the respective infection probabilities $p_q^{(strain)}$ are calculated for all quarters q every day/time step, depending on the transmission mode, see equation (1). In the equation, all parameters except the susceptibility factor $Susc_q$ (see II.2.2) and the total number of quarters N depend on the pathogen strain, which is not specifically notated in (1) for easier readability, it will however be noted in the text (e.g., $\beta^{(strain)}$ instead of β).

$$p_q^{(strain)} = \begin{cases} 1 - \exp(-\beta \cdot Susc_q) & \text{(environmental),} \\ 1 - \exp\left(-\beta \cdot \frac{I}{N} \cdot Susc_q\right) & \text{(contagious),} \\ 1 - \exp\left(-\beta \cdot \frac{(1-\varepsilon)\cdot I + \varepsilon\cdot\eta\cdot\left(\frac{\sum_{i=1}^{d} 0.01^{i\cdot d^{-1}}\cdot I_i}{\sum_{i=1}^{d} 0.01^{i\cdot d^{-1}}} + \theta\right)}{N} \cdot Susc_q\right) & \text{(opportunistic).} \end{cases} \tag{1}$$

$\beta^{(strain)}$ is the transmission rate of that pathogen strain. For environmental strains, the infection probabilities only depend on the respective transmission rates. $I^{(strain)} = I_S^{(strain)} + I_C^{(strain)}$ (see Figure II.1) is the number of quarters (in lactating cows) already infected with a specific pathogen strain at the beginning of the current time step t_0. A higher number of infected quarters $I^{(strain)}$, increases the infection probability for contagious and opportunistic strains. $I_i^{(strain)}$ is the number of quarters that were infected with a strain at the end of time step $t_0 - i$. It is taken into account in the environmental part of opportunistic transmission, where the pathogen strain decays in the environment for $d^{(strain)}$ days until 1% of the initial bacteria remain and then disappear. The environmental share is given by $\varepsilon^{(strain)}$, while $\eta^{(strain)}$ is an additional scaling factor for the infectiousness of the strain's environmental part compared to its contagious part. $\theta^{(strain)}$, representing a purely environmental factor (e.g., introduction by humans), allows (re)infection with a strain that is not present in the cows nor the environment of the herd. All parameter values can be found in Table II.1.

The probabilities $p_q^{(strain)}$ of the active pathogens are combined by

$$p_q^{(total)} = 1 - \prod(1 - p_q^{(strain)}), \tag{2}$$

and each previously uninfected quarter gets infected with this probability. The infecting pathogens are then drawn according to their relative risk. Infected quarters are allocated to $I_S^{(strain)}$ or $I_C^{(strain)}$ depending on the probability $P_c^{(strain)}$ (Table II.1).

At each new time step, previously subclinically infected quarters in I_S have the chance to flare up or spontaneously recover (Figure II.1) with a certain pathogen-specific probability (Table II.1). The clinically infected quarters are subjected to a three day

Parameter	Description	Pathogen	Value	Reference
Transmission rate (β)	Rate for susceptible animals entering subclinical or clinical state	*S. aureus*	0.0179*	Zadoks et al. (2002)
		S. agalactiae	0.0068	Leelahapongsathon et al. (2016)
		S. uberis (contagious)	0.0155*	Zadoks et al. (2001a)
		S. uberis (environmental)	0.0155*	Zadoks et al. (2001a)
		E. coli	0.0001*	Barkema et al. (1998)
Probability of clinical state (P_c)	Probability of entering clinical state when infected	*S. aureus*	0.17	Swinkels et al. (2005a)
		S. agalactiae	0.01*	**
		S. uberis (contagious)	0.32	Zadoks et al. (2003)
		S. uberis (environmental)	0.32	Zadoks et al. (2003)
		E. coli	0.85	Hogan and Smith (2003)
Flare up probability	Probability of subclinical animals going to clinical state	*S. aureus*	0.0081*	Swinkels et al. (2005a)
		S. agalactiae	0.0005*	**
		S. uberis (contagious)	0.0068*	Swinkels et al. (2005b)
		S. uberis (environmental)	0.0068*	Swinkels et al. (2005b)
		E. coli	0.0035*	Döpfer et al. (1999)
Spontaneous recovery probability	Base probability of spontaneous cure for subclinical animals	*S. aureus*	0.0064*	van den Borne et al. (2010b)
		S. agalactiae	0.0023*	Leelahapongsathon et al. (2016)
		S. uberis (contagious)	0.0143*	van den Borne et al. (2010b)
		S. uberis (environmental)	0.0143*	van den Borne et al. (2010b)
		E. coli	0.0221*	van den Borne et al. (2010b)
Recovery probability	Probability of recovery for clinical animals that are treated	*S. aureus*	0.4	Steeneveld et al. (2011)
		S. agalactiae	0.7	Steeneveld et al. (2011)***
		S. uberis (contagious)	0.7	Steeneveld et al. (2011)
		S. uberis (environmental)	0.7	Steeneveld et al. (2011)
		E. coli	0.8	Steeneveld et al. (2011)
ε	Environmental share in opportunistic transmission	*S. agalactiae*	0.1	arbitrary
η	Scaling factor for infectiousness of environmental part in opportunistic transmission	*S. agalactiae*	1	arbitrary
θ	Purely environmental factor in opportunistic transmission	*S. agalactiae*	0	arbitrary
d	Number of days the bacteria survive in the environment	*S. agalactiae*	40	arbitrary, more than four weeks (Jørgensen et al., 2016)

* rounded values
** *S. aureus* values were adjusted by the factor by which incidence is different in Barkema et al. (1998).
*** used value of *Streptococcus dysgalactiae* or *uberis*

Table II.1: Rates and probabilities used in the transmission framework for lactating cows (Figure II.1). All parameters are implemented in daily time steps, for all quarters.

Parameter	Description	Pathogen	Value	Reference
Transmission rate (β_{dry})	Rate for susceptible animals entering subclinical or clinical state	*S. aureus*	0.0179*	**
		S. agalactiae	0.0011*	***
		S. uberis (contagious)	0.0011*	Halasa et al. (2010, 2009c)
		S. uberis (environmental)	0.0011*	Halasa et al. (2010, 2009c)
		E. coli	0.0001*	**
Transmission rate ($\beta_{dry}^{(dct)}$)	Rate for susceptible animals with dry cow treatment entering subclinical or clinical state	*S. aureus*	0.0005*	Halasa et al. (2010, 2009c)
		S. agalactiae	0.0003*	***
		S. uberis (contagious)	0.0003*	Halasa et al. (2010, 2009c)
		S. uberis (environmental)	0.0003*	Halasa et al. (2010, 2009c)
		E. coli	0.0001*	**
Probability of clinical state (P_c)	Probability of entering clinical state when infected	*S. aureus*	0.1	Halasa et al. (2010)
		S. agalactiae	0.1	***
		S. uberis (contagious)	0.1	Halasa et al. (2010)
		S. uberis (environmental)	0.1	Halasa et al. (2010)
		E. coli	0.1	Halasa et al. (2010)
Flare up probability	Probability of subclinical animals going to clinical state	*S. aureus*	0.006*	Halasa et al. (2010)
		S. agalactiae	0.0005*	**
		S. uberis (contagious)	0.004*	Halasa et al. (2010)
		S. uberis (environmental)	0.004*	Halasa et al. (2010)
		E. coli	0.0035*	**
Spontaneous recovery probability	Probability of spontaneous cure for subclinical animals	*S. aureus*	0.0079*	Halasa et al. (2010)
		S. agalactiae	0.0086*	***
		S. uberis (contagious)	0.0086*	Halasa et al. (2010)
		S. uberis (environmental)	0.0086*	Halasa et al. (2010)
		E. coli	0.0221*	**
Recovery probability	Probability of recovery for clinical animals with dry cow treatment	*S. aureus*	0.77	Halasa et al. (2010, 2009b)
		S. agalactiae	0.89	***
		S. uberis (contagious)	0.89	Halasa et al. (2010, 2009b)
		S. uberis (environmental)	0.89	Halasa et al. (2010, 2009b)
		E. coli	0.9	Halasa et al. (2010, 2009b)

* rounded values
** same value as during lactation
*** value of *Streptococcus* spp.

Table II.2: Rates and probabilities used in the transmission framework for dry cows (Figure II.1), probability of clinical state, flare up and spontaneous recovery were taken from Halasa et al. (2010), who recalculated them from Bradley and Green (2004) and Green et al. (2005). All parameters are implemented in daily time steps, for all quarters.

treatment (default) with antibiotics, they will thereafter either recover or persist as subclinical cases (remission) (Figure II.1). The probability for recovery depends on the causative pathogen and is cow-specific, according to Steeneveld et al. (2011) (Table II.1 shows the base probability).

The model includes the possibility to scale transmission rate, flare up probability, and spontaneous recovery probability by any factor, and to replace the probability $P_c^{(pathogen)}$ that a new infection will be clinical by another value.

Dry cows

For dry cows, IMI will generally be or stay subclinical, except in the first or last week of the dry period, where clinical IMI can also occur.

New infections in dry cows can occur for every active strain. Contagious strains are considered active, if at least one quarter of one cow in the herd is infected with that particular strain. Similarly, opportunistic strains are considered active, if they are still present in the herd or if they have a non-zero purely environmental element $\theta^{(strain)}$. This is important, as the probability of infection is calculated according to

$$p^{(strain)} = 1 - \exp\left(-\beta_{dry}^{(dct,strain)}\right) \tag{3}$$

for every active pathogen strain, where $\beta_{dry}^{(dry,strain)}$ is depending on both the pathogen and whether the cow was treated with dry cow therapy or not (Table II.2). Note that the infection probability in the dry period is the same for all quarters and not cow-specific. The probabilities of the active pathogens are combined by (2). Each previously uninfected quarter gets infected with this probability. The infecting pathogens are then drawn according to their relative risk. Infected quarters are allocated to $I_S^{(strain)}$ or, if the cow is in the first or last week of the dry period, to $I_S^{(strain)}$ or $I_C^{(strain)}$ depending on the probability $P_{c,dry}^{(strain)}$ (Table II.2).

Similar to lactating cows, subclinically infected quarters in dry cows can flare up or spontaneously recover (Table II.2), however flare up can only happen in the first or last week of the dry period.

Additionally, a cow with a flared up quarter in the first week after dry off will receive dry cow treatment. Dry cow quarters change their status from I_C to I_S or S after the same number of days as for clinical cases in lactating cows. Here, dry cow treatment influences the probability of recovery for the clinical quarter (Table II.2). For clinical quarters in the last week of the dry period, the probability for recovery is calculated similarly as for lactating cows, only without regarding somatic cell count (SCC) and days in milk.

As for lactating cows, transmission rate, flare up probability, and spontaneous recovery probability can be scaled and the probability $P_{c,dry}^{(strain)}$ that a new infection will be clinical can be replaced.

Heifers

Currently, there is no dynamic pathogen transmission in heifers, i.e. cows prior to their first calving. Instead, each pathogen strain has a certain probability to infect heifers (Table II.2). These probabilities are added up for the active pathogens to a total probability for heifers to be assigned to I_S one day before calving. The pathogens are then drawn depending on their part of the total probability.

II.2.3 Production effects and economy

Feeding

Cows are often fed roughage as basic feed plus concentrate to facilitate a higher milk production. To our knowledge, no studies have explicitly estimated the decrease in feed due to IMI. In this model, the feed usage per lactating cow is a function of the energy-corrected milk (ECM) produced, corresponding to €0.1852 per ECM (following Kirkeby et al., 2016). Therefore, cows with subclinical and clinical IMI automatically have a decreased feed intake because their milk production is decreased, as described below in sections II.2.3 and II.2.3. The model also includes an additional option to simulate a farmer who feeds only roughage without concentrate to cows that have their milk withdrawn due to antibiotic treatment, as described in Halasa et al. (2009a). For those cases, a proportion of the feed costs is subtracted to account for lower concentrate usage. By default, however, this option is disabled.

Milking

The daily milk yield is calculated for lactating cows (Kirkeby et al., 2016). However, differing from the iCull model, the income from milk is now dependent on the fat and protein content. Using the

data set described in Kirkeby et al. (2016), we estimated the daily mean protein percentage for all cows, depending on days in milk (DIM) and parity (1, 2 and 3+), and fitted a three parameter Wood curve to each cow and parity in the data set (see Græsbøll et al., 2016). Based on the same data set, we estimated distributions for the fat to protein ratio per parity. In the simulation model, each cow is assigned three parameters for the Wood curve to describe the protein percentage and, for each simulated day, the protein content is calculated based on the cow's DIM. A fat to protein ratio is then drawn from the respective distribution for each cow and used to calculate the daily fat yield based on the milk yield and protein percentage.

The income from milk is given by summing the income from fat and protein, withdrawing a milk handling fee based on the daily kg milk yield and multiplying with a penalty or bonus factor, depending on the bulk tank SCC (Table II.3).

Parameter	Value	Description	Reference
Antibiotic treatment period	3	Number of days in antibiotic treatment, milk from treated cows is discarded.	Steeneveld et al. (2011)
Milk withdrawal period	6	Number of days after antibiotic treatment where the milk from treated cows is discarded.	van den Borne et al. (2010a), Michael Farre pers. comm.
Acute mastitis probability	0.01	Probability for a cow to get acute mastitis, when it gets clinical mastitis.	Michael Farre pers. comm.
Antibiotic treatment cost	€33.3	Cost for antibiotic treatment of one cow, not including vet visit or farmer labor.	Michael Farre pers. comm.
Dry cow therapy cost	€9.6	Cost for dry cow therapy for one cow, which includes teat sealants in 20% of the cases.	Michael Farre pers. comm.
Opportunity costs	€20	Opportunity costs for treatment of one cow with clinical mastitis.	Halasa et al., 2009a, Michael Farre pers. comm.
Protein price Fat price Milk handling fee	€5.8132 €4.1519 €0.0001343	Price for 1 kg protein Price for 1 kg protein Fee for handling 1 kg milk	www.arla.dk, September 2017 www.arla.dk, September 2017 www.arla.dk, September 2017
Culling costs	ca. €500	Costs for culling one cow (price of a new heifer minus slaughter value)	Huijps et al. (2008)

Table II.3: Model parameters related to IMI treatment, culling, and milk price.

Subclinical IMI

Subclinically infected animals are subject to an increased SCC. For every subclinical quarter, an increase in the SCC is added to the generic simulated SCC, according to Schepers et al. (1997, Table 1) and Wilson et al. (1997, Table 2, used for scaling missing pathogens). If more than one quarter of a cow is subclinically infected, the maximum increase is added; however, the SCC is cut off at a maximum of 10,000,000 as higher SCC values are rarely observed and for numeric stabilization. The increased SCC in these subclinically infected animals also leads to a higher bulk tank SCC, which is calculated as the weighted mean SCC in the total daily amount of milk produced. The milk price, in turn, is dependent on the bulk tank SCC, as a bulk tank SCC up to 200,000 will result in a 4% bonus, while a bulk tank SCC above 500,000 will result in the maximal penalty of 10% (see Kirkeby et al., 2016).

Linked to an increased SCC in subclinically infected cows is milk loss, and as the SCC varies daily for each cow, so does the milk loss. We used the estimates given in Hortet et al. (1999) to reduce the milk yield of each cow with at least one subclinical quarter according to SCC, DIM (except for primiparous cows) and parity (primiparous or multiparous, where we used the estimates for parity 1 or 3+ cows, respectively), see supplementary Figure II.S7. The milk loss per cow is restricted to 2kg, which corresponds to the maximal loss in parity 3+ within the limits of Hortet et al. (1999).

Clinical IMI

Clinical mastitis can reduce a cow's milk production even after the cow is not clinical anymore (Gröhn et al., 2004). We used Gröhn et al. (2004) estimates for milk loss following clinical infection to fit logarithmic functions to the amount of milk lost for each pathogen type and for primiparous and multiparous cows. As a logarithmic function did not seem to be a suitable fit for primiparous cows with clinical IMI caused by *Streptococcus* spp., we fitted in this case a linear function truncated at zero (Figure II.S9). The respective milk loss is added throughout the whole lactation to the cow's produced milk, starting on the first

day of clinical infection. An example of the milk loss is given in Figure II.S8.

When a cow gets a clinical IMI, it is treated. In the model, the default option comprises three days of antibiotic treatment, during which and for six days afterwards the cow's milk is withdrawn and discarded (Table II.3). Treatment costs are based on expert opinion on Danish herds (see Table II.3) and are comparable to the numbers given in Halasa et al. (2009a). They are divided into the costs of the antibiotics (€33.3) and opportunity costs (€20), which include the time the farmer has to spend on cows with clinical IMI (Table II.3).

Dry cow therapy

Dry cow therapy is the treatment of cows with long lasting antibiotics at dry off. In the model, the default option applies antibiotic treatment only to cows that get a clinical IMI during the first week of dry off to study the dynamics of IMI without the influence of specific dry cow management. Other options include different selection strategies for selective dry cow therapy: cows with a history of clinical IMI, cows with a high SCC at the last monthly milk yield recording, cows with either of those options, and blanket dry cow therapy.

Culling

In the model, culling happens on a weekly basis. If there are more than 200 lactating cows, the farmer will cull the excess number of cows. About half of the culled cows are chosen randomly, those are the cases that have to be culled e.g. because of lameness. The others are chosen by the farmer from a culling list, where (s)he prioritizes the animals for culling, e.g. cows with production in the bottom 20%, with insemination difficulties, or with a high SCC at the last monthly milk yield recording, by applying weights for every unfavorable circumstance to each cow (Kirkeby et al., 2016). For every high SCC ($> 200,000$) at subsequent monthly milk yield recordings, the respective cows will be increasingly prioritized, with a low SCC resetting this prioritization. After 12 months with a continuously high SCC, cows will be culled at the first possibility, though the default

value of 12 months can be easily changed to reflect different management strategies.

As subclinical IMI causes an increase in the SCC, cows with subclinical IMI have a higher probability to be prioritized for culling because of a high SCC. Cows with previous clinical IMI also have a higher probability of being chosen for culling than their herd mates. Bar et al. (2008a) found that the odds ratios for primiparous cows being culled were 7.46, 16.12 and 20.08, if they had 1, 2 or $\geqslant 3$ clinical mastitis cases, respectively (exponentiating values of Table 4 in Bar et al., 2008a); for multiparous cows the respective odds ratios were 3.74, 5.00 and 6.36. We used these values to apply weights to the culling decision made by the farmer, with multiparous cows with one previous clinical IMI receiving a weight of 1 and the other mentioned cases receiving weights scaled to reflect the ratios found in Bar et al. (2008a). Furthermore, it can happen, that a cow gets flagged for an acute IMI when it becomes clinical (Table II.3). These cows will be put on top of the culling list, from which the farmer chooses in the weekly culling.

Prioritization for culling is therefore: involuntary cases, cows with acute IMI, cows with a continuously high SCC, cows with the highest weight for culling.

The costs of culled animals are calculated as the costs for raising a replacement heifer for each cow that is culled (to two years of age, €510), minus the slaughter value the farmer gets for the culled cow (€51).

Model outputs

The epidemiological model output consists of daily cow level prevalences, as well as the total number of flare ups, remissions, subclinical, and clinical IMI for each simulation. Furthermore, it includes the total number of culled cows due to acute IMI, subclinical IMI and a history of clinical IMI. Culling due to subclinical IMI includes all culled cows with at least one subclinically infected quarter that were prioritized for culling because of a high SCC. To avoid counting a culled cow several times, culling due to the cow having a history of clinical IMI includes only cases in which the cow did not have a high SCC at the last monthly milk

yield recording. Cows culled because of acute IMI are counted separately.

The economical model output includes the total milk loss in kg due to subclinical or clinical IMI (both milk loss and withdrawal), as well as the total income from milk and the mean milk price penalty percentage (see sections II.2.3, II.2.3, and II.2.3). As the fat and protein percentages for lost milk are not calculated, a mean milk price of around €0.4099 per kg is used to calculate costs for milk loss (mean Arla milk price in September 2017). The mean milk price penalty value together with the total income from milk is used to calculate the possible penalty paid due to a high bulk tank SCC. Further economic output includes expenses for treatment of clinical IMI as described above (II.2.3), as well as costs for dry cow therapy (Table II.3).

II.2.4 Model validation

Several methods for internal validation were used on the model (Sargent, 2003). *Rationalism*, including *operational graphics*: various scenarios were compared to check consistency and credibility of model outputs. *Traces*: single cows were traced over time to check for consistency. *Face validity*: model assumptions and outputs were evaluated by mastitis experts. External validation was conducted by comparing model predictions to the literature, as data to validate such a complicated model is not available.

Model convergence

We tested model convergence on two parameters by simulating 1000 iterations. In a scenario without any IMI causing pathogens, we tested convergence of the energy corrected milk yield (ECM), and in a scenario with three pathogens (using default parameters taken from literature, see Table II.1), we tested convergence on the number of clinical cases. In both cases visual inspection showed that 500 iterations were sufficient to obtain stable results (Figure II.S6). Further visual inspections showed that after five simulated years herd, population, and transmission dynamics were always stable, warranting a five year burn-in period.

Sensitivity analysis and model runs

We performed sensitivity analysis on a herd with 200 cows, using 500 simulations of 5 years, with a burn-in time of an additional 5 years. All scenarios were initiated with a 20% starting prevalence for all pathogen strains, with the exception of environmental strains in multiple pathogen scenarios, where the starting prevalence was set to 10%. These values are arbitrary and were chosen only for presentation of the model.

Sensitivity analyses were carried out on the transmission rate (β), the spontaneous recovery probability, the probability that a newly infected animal becomes a clinical IMI case (P_c), the flare up probabilities, the environmental part of the opportunistic pathogen (ε), and the number of days the opportunistic pathogen can survive in the environment (d). For the transmission rate parameter, various scaling factors (all < 1, except for *E. coli* where factors > 1 were considered) were considered in the sensitivity analysis; selected values are presented in Table II.4. Sensitivity analysis for the spontaneous recovery probability consisted of several scaling factors between 0.25 and 2. For the other parameters, sensitivity analyses focused on the actual value instead of the scaling factor: P_c and flare up probability were varied between 0.01 and 0.85 and between 0.0002 and 0.02, respectively. The parameter ε in opportunistic transmission was varied between 0 and 1, while d was reduced down to 10 days in increments of 5 days.

To obtain insight into how the model would simulate the dynamics of pathogen spread, a great number of scenarios were run in the sensitivity analyses, of which only a few with different transmission rates were selected and presented here. In the supplementary material, more scenarios were included.

II.3 RESULTS

The methods used for internal validation showed valid and consistent outcomes of the model in all scenarios. As an illustration, nine scenarios were selected. These include four one pathogen scenarios for different pathogens, four two pathogen scenarios

Scenario	Pathogens	Transmission rate scaling factor	Transmission rate	Transmission rate (dry cows)
7	*S. aureus*	0.2	0.003,6	0.003,6
9	*S. aureus*	0.1	0.001,8	0.001,8
14	*S. agalactiae*	0.55	0.003,7	0.000,6
21	*S. uberis* (contagious)	0.8	0.012,4	0.000,9
49	*S. aureus*	0.25	0.004,5	0.004,5
	S. agalactiae	0.5	0.003,4	0.000,6
56	*S. aureus*	0.25	0.004,5	0.004,5
	S. uberis (contagious)	0.5	0.007,8	0.000,6
68	*S. agalactiae*	0.5	0.003,4	0.000,6
	S. uberis (contagious)	0.5	0.007,8	0.000,6
88	*S. uberis* (contagious)	0.5	0.007,8	0.000,6
	S. uberis (environmental)	0.01	0.000,2	0.000,01
98	*S. aureus*	0.25	0.004,5	0.004,5
	S. agalactiae	0.5	0.003,4	0.000,6
	S. uberis (contagious)	0.5	0.007,8	0.000,6
	S. uberis (environmental)	0.01	0.000,2	0.000,01
	E. coli	1	0.000,1	0.000,1

Table II.4: Selected scenarios. All pathogens start with a 20% prevalence, except in the five pathogen scenario (98), where the environmental strains start with a 10% prevalence. Transmission rates are rounded.

with different pathogen combinations, and one five pathogen scenario with all pathogens (Table II.4). Scenarios where exact literature values were used as transmission parameters, led to high prevalences in our setting (results not shown). Therefore, the selected scenarios used adjusted transmission rates, leading to more realistic prevalence estimates (Figures II.2, II.3, and II.4).

Figure II.2 shows selected scenarios with one active pathogen. The starting prevalence is set to 20%, and during the burn-in period it fluctuates depending on the pathogen strain (i.e. the combination of all transmission parameters, see scenarios 7, 14, and 21, Figure II.2), or changes depending on the transmission rate (scenarios 9 and 7, Figure II.2). After the burn-in period, the prevalence has reached a mostly stable level.

The model also allows coexistence of multiple pathogens or strains, regardless of their transmission mode, on different prevalence levels, depending on the scaling of the transmission parameters (Figures II.3 and II.4). Scenario 49 and 56 show two pathogen scenarios, where the active pathogen strains are *S. aureus* and *S. agalactiae* or a contagious *S. uberis*, respectively. The transmission rate for *S. aureus* is the same in both scenarios, however the mean daily prevalence is higher in scenario 56, where the second active pathogen is present at a low level. In scenario 68, *S. agalactiae* and the contagious *S. uberis* strain are coexisting

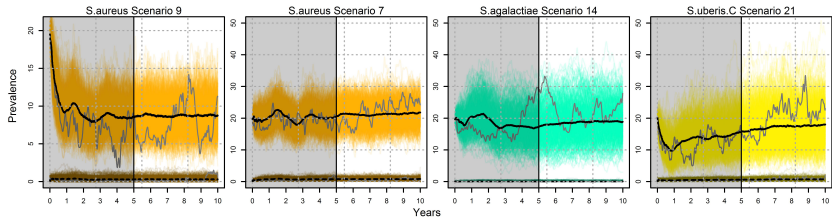

Figure II.2: Cow level prevalences in one pathogen scenarios 7, 9, 14, and 21, see Table II.4. Every scenario shows smoothed daily prevalences for each of 500 iterations over 5 years with a 5 year burn-in period (with one random iteration displayed in gray), as well as the mean daily prevalence (bold, black). The bottom lines show the daily prevalences of clinical IMI, with the mean displayed as a dashed line.

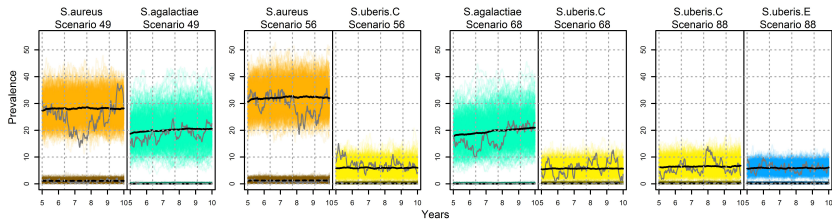

Figure II.3: Cow level prevalences in two pathogen scenarios 49, 56, 68, and 88, see Table II.4. Every scenario shows smoothed daily prevalences for each of 500 iterations over 5 years after a 5 year burn-in period (with one random iteration displayed in gray), as well as the mean daily prevalence (bold, black). The bottom lines show the daily prevalences of clinical IMI, with the mean displayed as a dashed line.

at similar levels to scenario 49 and 56, respectively. Scenario 88 (Figure II.3) shows another scenario with two active pathogen strains, in this case a contagious and an environmental strain of *S. uberis*. Both strains are present at a similar prevalence, with a higher variation for the contagious strain.

Scenario 98 shows a five pathogen, which also includes both a contagious and an environmental strain of *S. uberis* together with contagious *S. aureus*, environmental *E. coli*, and opportunistic *S. agalactiae* (Figure II.4). The contagious strains have the same transmission rate as in the two pathogen scenarios and are at similar daily prevalence levels. The opportunistic *S. agalactiae*

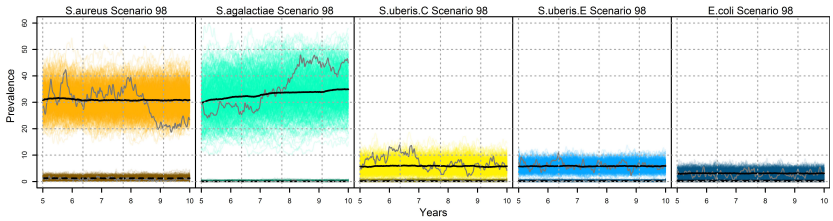

Figure II.4: Cow level prevalences in the five pathogen scenario see Table II.4, scenario 98. It shows the smoothed daily prevalences for each of 500 iterations over 5 years after a 5 year burn-in period (with one random iteration displayed in gray), as well as the mean daily prevalence (bold, black). The bottom lines show the daily prevalences of clinical IMI, with the mean displayed as a dashed line.

Scenario	Flare up		Remission		subclinical IMI		clinical IMI	
7	120	(101, 140)	113	(95, 134)	258	(228, 293)	134	(113, 157)
9	44	(36, 53)	40	(32, 48)	98	(84, 112)	47	(38, 57)
14	7	(4, 10)	4	(2, 6)	77	(51, 107)	8	(5, 12)
21	78	(57, 114)	72	(49, 110)	244	(174, 363)	149	(105, 225)
49	172	(147, 200)	164	(138, 192)	436	(385, 498)	196	(167, 227)
56	221	(197, 257)	209	(186, 247)	482	(437, 551)	264	(237, 306)
68	33	(27, 40)	22	(17, 28)	139	(112, 171)	46	(38, 56)
88	58	(50, 67)	45	(37, 53)	136	(120, 156)	94	(82, 108)
98	261	(232, 294)	250	(221, 284)	693	(640, 752)	370	(333, 410)

Table II.5: Epidemiological output in median number (with 5% and 95% percentiles) of quarter cases per year (mean over 5 years) of the scenarios in Table II.4. Numbers are rounded.

strain also has the same transmission rate as in the two pathogen scenarios, but the prevalence level has increased.

The epidemiological output in Table II.5 shows the number of quarter cases per year (median over 500 iterations and mean over 5 years simulation period); in the multiple pathogen scenarios, numbers are summed over all pathogens. The number of subclinical IMI includes all remission cases, while the number of clinical IMI includes all flare up cases. Also, one cow may be counted more than one time for the same infection, e.g. if a clinical quarter went into remission and flared up again afterwards. For *S. agalactiae*, most clinical IMI are flared up subclinical cases and there are few clinical IMI, if the prevalence is at moderate levels (scenario 14). In contrast, the contagious *S. uberis* strain leads to many more clinical cases, both flared up and directly infected (scenario 21).

Scenario	high SCC		acute IMI		history of IMI	
7	22	(18, 26)	1	(0, 2)	3	(2, 4)
9	13	(10, 16)	0	(0, 1)	2	(1, 3)
14	24	(19, 29)	0	(0, 0)	0	(0, 1)
21	15	(10, 20)	1	(0, 2)	4	(3, 6)
49	48	(41, 55)	1	(1, 2)	2	(1, 3)
56	35	(30, 39)	2	(1, 3)	3	(2, 4)
68	27	(21, 33)	0	(0, 1)	2	(1, 3)
88	10	(7, 13)	1	(0, 1)	5	(3, 6)
98	68	(60, 76)	2	(1, 3)	2	(1, 4)

Table II.6: Median number (with 5% and 95% percentiles) of culled cases per year (mean over 5 years) of the scenarios in Table II.4, see section II.2.3 for culling categories. Numbers are rounded.

Table II.6 shows the number of culled cows per year (median over 500 iterations and mean over 5 years simulation period); acutely culled cows, subclinical cows that were culled with a noticeable high SCC, and culled cows with a history of clinical IMI. Most cows that are culled because of IMI related reasons are connected to a high SCC, though the number and proportion of cows culled with subclinical IMI or a history of clinical IMI also depends on the causative pathogen.

The costs associated with subclinical and clinical IMI can be found in the supplementary Tables II.S1, II.S2, and II.S3.

Further sensitivity analyses showed that the probability of spontaneous recovery for subclinical cases is similarly influential on the prevalence as the transmission rate (Figure II.S1): with a higher probability of spontaneous recovery, the prevalence decreases.

Sensitivity analysis for the probability for a newly infected quarter to be clinical (P_c, Table II.1) showed that for *S. agalactiae* and the contagious strain of *S. uberis* an increased proportion of clinical cases leads to a decreased prevalence, while this effect was less observable in the environmental pathogens and *S. aureus* (Figure II.S2). Similarly, the higher the flare up probability is, the lower the prevalence becomes (Figure II.S3).

If the environmental part ε in the opportunistic infection is increased, the prevalence increases, too, ranging from a mean of 17.8% with pure contagious infection ($\varepsilon = 0$) to a mean of 30.3 without any contagious part ($\varepsilon = 1$) after 10 years. This effect is not visible, if the prevalence is low ($< 5\%$, Figure II.S4).

Reducing the number of days d the opportunistic pathogen can survive in the environment showed marginal effects on model outcome, which is expected due to how the bacterial survival is weighted.

II.4 DISCUSSION

The described model simulates a dairy cattle herd on daily basis, including the spread of IMI causing pathogens within the herd. It also includes various treatment variants for IMI, that will be investigated for cost-effectiveness in following studies. The aim of this study was to describe the model, including a new opportunistic transmission mode. With the historical view of purely contagious and purely environmental pathogens being questioned (e.g., Jørgensen et al., 2016; Zadoks et al., 2001a), we think that this feature is an important step in modelling IMI spread, representing both the possibility of strain specific transmission properties and recent suggestions of *S. agalactiae*'s potential opportunistic behaviour. The opportunistic transmission mode combines both contagious (via milking) and environmental transmission in one strain, as indicated by Jørgensen et al. (2016). The contagious part of this new feature was transferred from previously already implemented contagious transmission (Halasa et al., 2009a). The environmental part represents the decay of the pathogen in the environment over time. Using an exponential function to represent the decay of infectious agents in the environment is not unusual, although slope of decline may differ for different pathogens (e.g., Halasa et al., 2016; Whiting et al., 1996). Still, implementing it for *S. agalactiae* should be acceptable. The infection probability depends on three main elements, in addition to the basic transmission rate; the contribution of the environment, the slope of decay, and the duration of pathogen survival in the environment. The latter has been approximated based on literature (Jørgensen et al., 2016), but the two other elements are lacking actual data from the field. We speculate that the impact of the environmental part is strain-specific, meaning that some strains are mainly found in the environment, persisting there for some time and causing new infections, while others

mainly spread via the contagious route. The weight of each mechanism is unknown, which warrants further research to assess the influence of the environment on the spread of this pathogen. Our model allows weighting of the contagious and environmental parts of pathogen spread, depending on, e.g., the strain type. Our current parameterization ($\varepsilon = 0.1$) would represent a mainly contagious spread of the pathogen with occasional transmission of IMI through the environment. This was done as an illustration of opportunistic spread in the model. In the future, the effect of the different parameters must be examined properly, when simulating the impact of control strategies against IMI caused by *S. agalactiae*.

Default values for all transmission parameters were taken from literature (Tables II.1 and II.2), which led to unrealistically high prevalences (Figures II.2, II.3, and II.4). This is not surprising, as studies are usually conducted in herds with large problems or even outbreaks with the specific pathogens. In those herds, pathogen spread, and thereby the calculated transmission rates, are high. On top of that, our additional susceptibility factor $Susc_q$, used to re-scale the transmission rates to include cow-specific infection, leads to higher infection probabilities in the model. The estimated transmission rates from the literature do not consider quarter factors, but instead they are average values for all quarters. For instance, it is known that the risk of infection is higher for quarters with previous IMI (Zadoks et al., 2001b). In order to consider the effect of these factors in the estimation of infection probability, the transmission rates are multiplied by the relative risks of quarter factors (the susceptibility factor), and hence the probability becomes artificially higher than normal. To represent a realistic situation, it therefore becomes important to rescale the transmission rates, as the quarter factors should be taken into account at the same time. Future studies estimating transmission rates should consider the effects of quarter and cow factors on the transmission rate, if possible, in order to be able to accurately model spread dynamics of IMI causing pathogens.

Our results show sensitivity of the prevalence to changes in transmission rate and other transmission parameters (see Figures II.S1–II.S4), making the use of the right parameters important. It is therefore worrisome that estimates of transmission rates are

scarce and limited to few studies from few herds (e.g., Barlow et al., 2013; Leelahapongsathon et al., 2016; Zadoks et al., 2001a, 2003). Nevertheless, we decided to include a susceptibility factor and thereby cow-specific transmission in the model and adjust transmission, as studies have shown that relevant risk factors exist (e.g., Zadoks et al., 2001b). As these factors may be pertinent for management decisions regarding IMI, not including them would prevent investigating cow-specific management strategies in the future. Furthermore, as IMI causing pathogens are thought to be transmitted, among other things, during the milking process (Harmon, 1994) or through reservoirs in the environment (Blowey and Edmondson, 2010; Zadoks et al., 2001a), transmission rates are dependent on herd related factors. Considering that there are only few studies estimating these rates, and conditions are prone to change over the years, transmission rates, in the absence of proper data, will have to be adjusted in some way to model different IMI situations representing different herds or management systems. Hopefully, future research can close these gaps.

Another point regarding transmission is the assumption in the model that the same transmission rate can be used for transmission to quarters of the same cow or of other cows. When transmission happens through the milking equipment, for instance, fluctuations in the milking vacuum could, depending on the milking machine's claw, lead to a reflux of milk from an infected quarter into uninfectedbteats (Besier et al., 2016). IMI can also be transmitted by flies (Owens et al., 1998). A fly would probably land on a quarter of the same cow before flying away, possibly leading to a higher risk of within cow spread. Given the absence of proper data to parameterize this process, the made assumption seems inevitable. Should this knowledge gap be closed in the future, different transmission probabilities could be used for within cow and between cow transmission.

Our results showed expected behavior when parameters were changed in sensitivity analyses. Different scenarios showed different prevalence patterns, e.g., in scenarios 49 and 56 (Figure II.3), where the prevalence of *S. aureus* was higher when the second pathogen's prevalence was lower. In scenario 98 (Figure II.4), *S. agalactiae* reached a higher prevalence level than in scenarios

49 or 68 (Figure II.3), even though the transmission rate was the same, showing a different behavior of opportunistic transmission depending on the prevalence of other IMI pathogens. An increased total prevalence leads to more quarters having a higher risk of contracting an IMI, as history of IMI is modelled as a risk factor for new IMI. This, combined with the fact that *S. agalactiae* can build up and persist in the environment, leads to its increased incidence. The model can thus simulate different transmission behaviors of pathogens and different herds, which is necessary to investigate, e.g., how effective a treatment regimen is under different circumstances. The economic part of the model yields comparable results to other models. For instance, Steeneveld et al. (2007) found an average per case cost of €109 for subclinical IMI, while our scenario 9 resulted in a median cost of about €100 per subclinical IMI case II.S1. In the same scenario, the median cost for a clinical IMI case was around €226, which is similar to other studies by e.g. Halasa et al. (2009a) (€101 to €328), Huijps et al. (2008) (€164 to €235), and Bar et al. (2008b) (€179 on average). As a substantial part of the costs for IMI arises from culling (Tables II.S1 and II.S2), and farmers behave differently in terms of culling (e.g., Fetrow et al., 2006), modelling herd-specific scenarios instead of average herds is also important for cost-effectiveness analyses.

Altogether, our model is able to simulate strain-, cow-, and herd-specific transmission of IMI causing pathogens on quarter level and with a daily time step. It also includes the possibility to consider different farmer priorities concerning culling by changing culling weights, or to include a prediction of the future value of a cow relative to its herd mates (Græsbøll et al., 2017) in the culling decision of the farmer, allowing a potentially more economic choice of cows to be culled. Moreover, the necessary features to study several treatment strategies for clinical IMI and selection strategies for dry cow therapy are already implemented in the model, and further strategies or pathogen strains can be easily added. This makes it possible to simulate specific herds and investigate the cost-effectiveness of various changes to management/prevention or treatment/control strategies, both short term (operational decision making) and long term (strategic decision making), that can also be strain- and cow-specific. As

different changes may be more cost-effective depending on the herd, and selective treatment decisions may be more effective when selecting the right cows to treat, it is important to include strain-, cow-, and herd-specifics in a model investigating cost-effective strategies. Simulating specific instead of average herds also means simulating diverse herd-specific disease situations, that are represented by different combinations of pathogens at different (stable) prevalence levels, which is possible with this model as shown in Figures II.3 and II.4.

Other bio-economic models simulating mastitis and mastitis control already exist, but to our knowledge none of the existing models combine all features presented in this model. The model by Halasa et al. (2009a, 2010) used the same transmission framework on cow instead of quarter level, but without including cow-specific infection and recovery. By simulating on quarter level, we allow multiple infections per cow, as this happens in reality, though one quarter can still only be infected by one pathogen or strain in our model. With this, our model also differs from e.g. the SIMMAST model (Allore et al., 1998), which also simulates on cow level, though it does not include cow-specific recovery, and only allows infection with one pathogen at a time. The SimHerd model (Østergaard et al., 2005) allows several pathogens per cow and is cow- and herd-specific, however, cow-specific factors are only considered for infection. SimHerd's mastitis framework is based on weekly time steps and infection through a baseline risk function for all mastitis pathogens. While this is a valid approach in the setting Østergaard et al. (2005) investigated, our model can explore both constant spread and infection over time, as well as transitions between the two. In addition, modelling on quarter level is closer to the underlying biology, as IMI occurs on quarter level. By modelling the actual biological unit, IMI management can also be modelled on quarter level, e.g. drying off chronically infected quarters.

While previous models may distinguish between contagious and environmental spread, our model explicitly allows both contagious and environmental strains of the same pathogen, exemplified by *S. uberis*, and also introduces a new third opportunistic transmission type with both contagious and environmental properties, as discussed above. Furthermore, while we only

included *S. uberis* with an environmental and a contagious strain to illustrate the possibility of having two strains with different transmission modes, this option should be kept in mind regarding other pathogens like e.g. *S. aureus*, as the model allows easy addition of other pathogens or pathogen strains. The question of which type of transmission is the right one for a particular pathogen strain cannot be answered by models, but our model allows the user to choose between the three mentioned transmission types and compare e.g. management strategies, depending on what kind of transmission is assumed for a strain. This allows investigations into cost-effectiveness of various strain-, cow-, and herd-specific IMI prevention and control measures, while including a farmer's current strategies, thereby hopefully making it easier to convince farmers to adopt proposed cost-effective changes in the future.

II.5 CONCLUSIONS

We developed a strain-, cow-, and herd-specific bio-economic simulation model of IMI and introduced a new opportunistic transmission mode. The model is sensitive to parameter changes in the transmission framework, but it can be fitted to simulate various pathogen scenarios, representing different herd situations. However, we found that available parameter estimations for IMI transmission or cure may be becoming outdated and we therefore suggest future studies to investigate new parameter estimations. The economic output allows cost estimations of both subclinical and clinical IMI, which lie within the ranges found in earlier studies. This makes it possible to use the model in future studies to investigate cost-effective prevention and control measures against IMI that are tailored to a specific herd, hopefully making it easier to convince the farmer to adopt proposed changes.

ACKNOWLEDGEMENTS

This study is part of the STOPMAST project, funded by the Danish Milk Levy Foundation (Mælkeafgiftsfonden), and part of

the EMCo-Mast project, funded by the Green Development and Demonstration Program (GUDP) under the Danish Directorate for Food, Fisheries and Agriculture, grant no. 34009-15-0918.

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SUPPLEMENTARY MATERIAL

Scenario	Milk loss		Milk penalty		Culling		Total		Per case	
7	9,955	(8823, 11087)	2,310	(1369, 3447)	10,006	(8170, 11842)	22,329	(19574, 24987)	86	(78, 94)
9	3,990	(3478, 4521)	−163	(-474, 152)	5,967	(4682, 7160)	9,799	(8153, 11293)	100	(86, 114)
14	12,357	(9070, 15927)	4,981	(2448, 7094)	10,832	(8537, 13316)	28,288	(20922, 34761)	369	(309, 442)
21	9,734	(7530, 12709)	3,206	(1537, 5405)	6,701	(4769, 9272)	19,761	(14264, 26978)	80	(69, 91)
49	24,551	(21767, 27476)	4,714	(3355, 6429)	21,940	(18911, 25061)	51,245	(45749, 57194)	118	(106, 131)
56	19,086	(17464, 20846)	4,750	(3377, 6448)	15,973	(13674, 18085)	39,758	(36308, 43533)	82	(75, 89)
68	16,015	(13306, 19175)	5,702	(3890, 7602)	12,301	(9639, 15055)	34,177	(28262, 40161)	244	(215, 277)
88	8,026	(7317, 8736)	1,992	(1263, 3068)	4,498	(3397, 5875)	14,533	(12699, 16760)	106	(96, 118)
98	38,592	(35651, 41668)	4,324	(3003, 5788)	31,212	(27627, 34976)	74,380	(68313, 80102)	107	(96, 118)

Table II.S1: Median costs (with 5% and 95% percentiles) in relation to subclinical IMI in € per year (mean over 5 years). Negative costs are benefits. Numbers are rounded.

Scenario	Milk loss		Treatment		Culling		Dry cow treatment		Total		Per case	
7	26,675	(18495, 37650)	6,867	(5713, 8016)	1,561	(1010, 2295)	58	(42, 75)	35,246	(27353, 46007)	267	(197, 356)
9	7,336	(4968, 10983)	2,412	(1908, 2878)	1,010	(551, 1561)	27	(15, 40)	10,779	(8285, 14629)	226	(173, 315)
14	389	(218, 678)	388	(213, 576)	184	(0, 367)	6	(0, 12)	949	(612, 1345)	121	(88, 185)
21	15,688	(8383, 28681)	7,843	(5488, 11890)	2,387	(1740, 3213)	18	(10, 31)	26,399	(18730, 39455)	175	(116, 281)
49	42,628	(30829, 58239)	10,120	(8485, 11673)	1,377	(826, 1928)	77	(56, 98)	54,005	(42375, 70358)	278	(209, 365)
56	63,613	(47700, 83022)	13,720	(12152, 15809)	2,111	(1469, 2938)	86	(65, 108)	79,662	(63689, 98681)	298	(236, 381)
68	4,614	(3021, 7418)	2,408	(1961, 2932)	1,102	(551, 1652)	13	(6, 21)	8,179	(6293, 11095)	175	(134, 244)
88	11,796	(8491, 16472)	4,950	(4317, 5673)	2,479	(1740, 3305)	10	(4, 17)	19,264	(15701, 24005)	206	(167, 263)
98	108,477	(89610, 127337)	19,278	(17373, 21260)	2,111	(1377, 2938)	88	(67, 113)	129,832	(111124, 148422)	351	(293, 411)

Table II.S2: Median costs (with 5% and 95% percentiles) in relation to clinical IMI in € per year (mean over 5 years). Numbers are rounded.

Scenario	Total	
7	57,346	(48622, 69180)
9	20,506	(17160, 24920)
14	29,295	(21675, 36026)
21	46,855	(34468, 63306)
49	105,616	(92116, 122999)
56	119,855	(102215, 139999)
68	42,555	(36345, 49014)
88	34,092	(29815, 39639)
98	203,787	(184474, 224119)

Table II.S3: Median total costs of IMI (with 5% and 95% percentiles) in € per year (mean over 5 years), see Tables II.S1 and II.S2. Numbers are rounded.

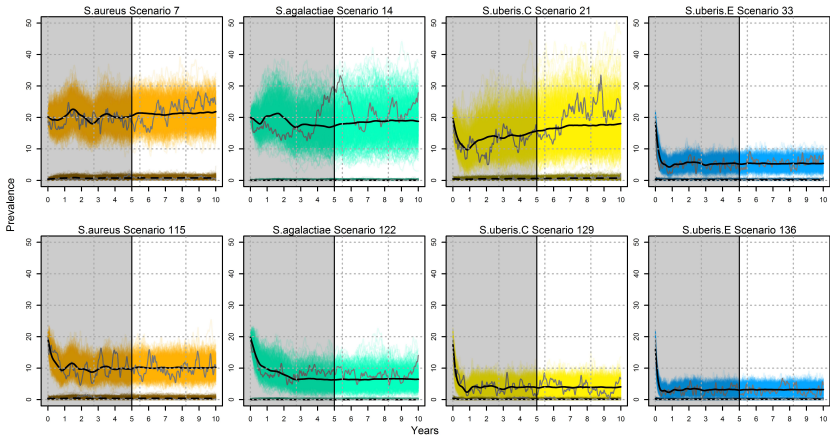

Figure II.S1: Sensitivity analysis for the spontaneous recovery probability. First row: cow level prevalences in one pathogen scenarios, see Table II.4 and Figure II.2. Second row: cow level prevalences in corresponding scenarios with doubled spontaneous recovery probability.

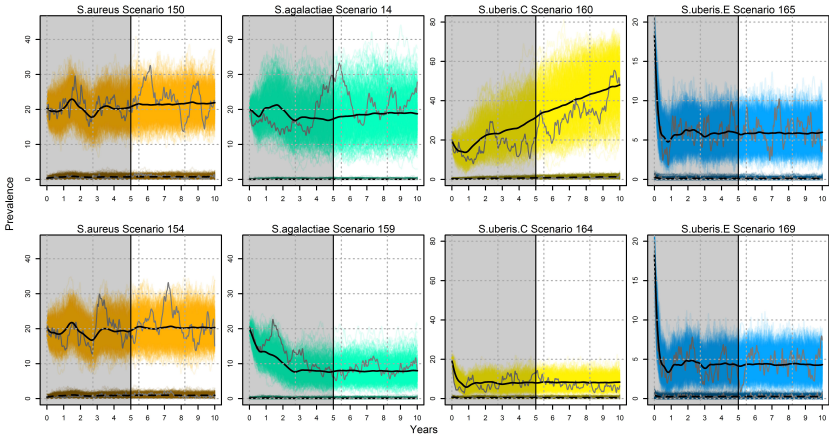

Figure II.S2: Sensitivity analysis for $P_c^{(pathogen)}$. Shown are cow level prevalences in one pathogen scenarios corresponding to scenarios 7, 14, 21, and 33 (see Table II.4 and Figure II.2) with a changed proportion of clinical cases. First row: $P_c^{(pathogen)} = 0.01$. Second row: $P_c^{(pathogen)} = 0.85$.

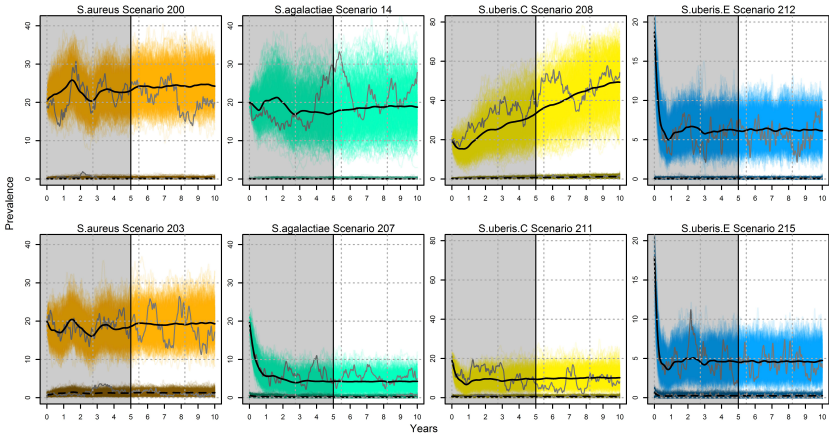

Figure II.S3: Sensitivity analysis for the flare up probability. Shown are cow level prevalences in one pathogen scenarios corresponding to scenarios 7, 14, 21, and 33 (see Table II.4 and Figure II.2) with scaled flare up probabilities. First row: flare up probabilities 0.0002 (*S. aureus*) or 0.0005. Second row: flare up probabilities 0.0163 (*S. aureus*), 0.0125 (*S. agalactiae*), or 0.0137 (*S. uberis*). Values are rounded.

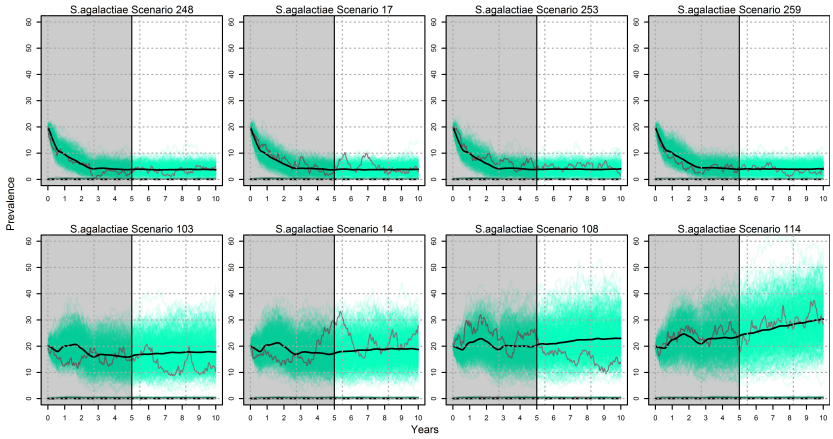

Figure II.S4: Sensitivity analysis of ε $(0, 0.1, 0.5, 1)$ at two different transmission rates (first row: 0.0017, second row: 0.0037).

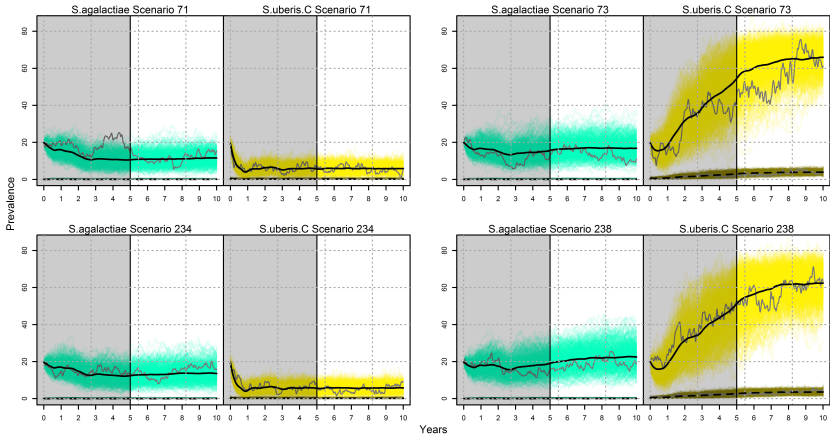

Figure II.S5: Influence of ε on pathogen dynamics in a two pathogen scenario. First row: scenarios 71 and 73, second row: scenarios corresponding to 71 and 73 with $\varepsilon = 0.9$.

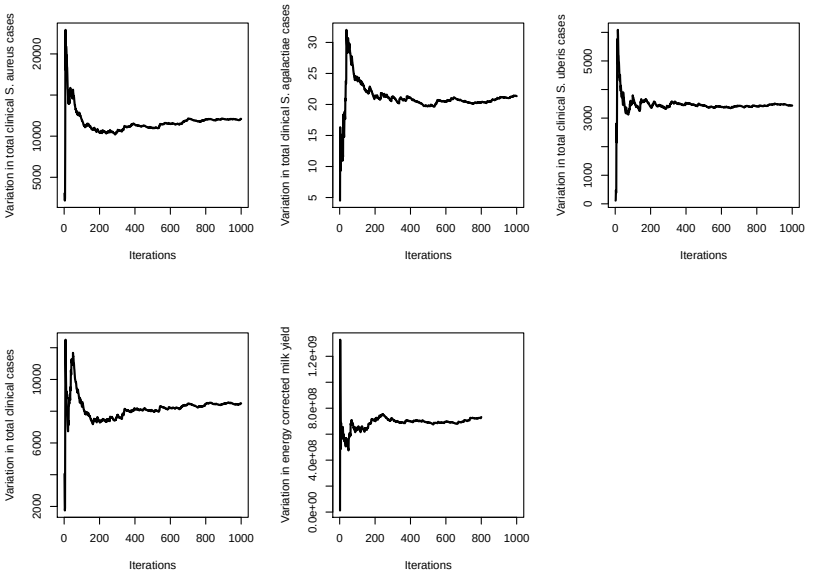

Figure II.S6: Model convergence. Upper row shows the variance in total clinical cases resulting from different numbers of iterations with single pathogen runs of *S. aureus*, *S. agalactiae*, *S. uberis* (environmental). Lower row shows the variance of clinical cases for the combined pathogens, and the variance in energy corrected milk yield (ECM).

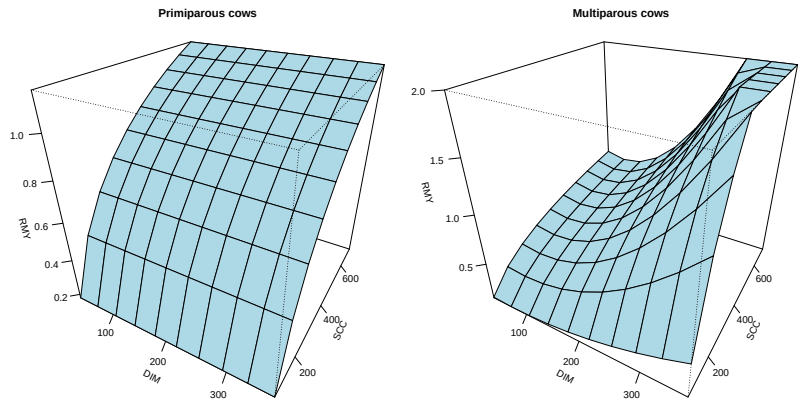

Figure II.S7: The functions for the reduction in the daily milk yield (RMY) in kg due to subclinical infection for individual cows based on the estimates from Hortet et al., 1999. In parity 1 the RMY is not dependent on DIM, which is the case for multiparous cows. Milk loss is cut at 2 kg.

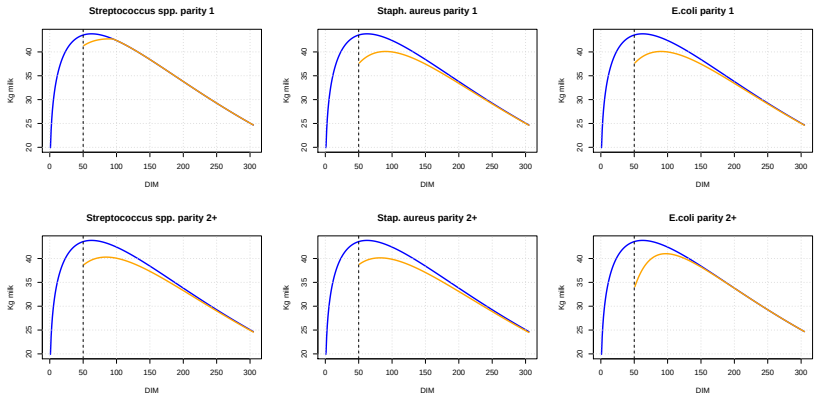

Figure II.S8: Examples of the milk loss following infection with three pathogen types in parity 1 or higher. The blue lines show a normal daily milk yield without daily variation. All examples are clinically infected at day 50 (dotted lines). The orange lines (starting at the dotted lines) show the milk yield after clinical infection and subsequent recovery towards the normal milk yield. The milk loss was estimated based on Gröhn et al., 2004

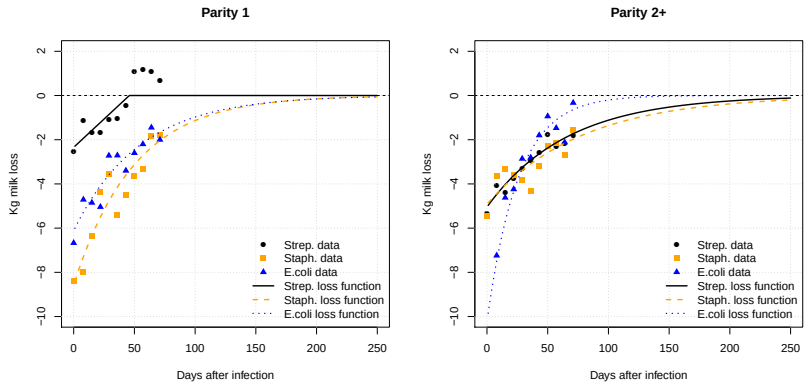

Figure II.S9: Fit of the milk loss functions used in the model based on data from Gröhn et al., 2004. Logarithmic functions were used for all pathogens except *Streptococcus* spp. in parity 1 where a linear function truncated at zero was used. The horizontal lines show zero milk loss.

4.4 MANUSCRIPT III

ECONOMIC AND EPIDEMIOLOGICAL IMPACT OF DIFFERENT INTERVENTION STRATEGIES AGAINST CLINICAL MASTITIS

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Submitted to *Journal of Dairy Science*

ABSTRACT

The overall aim of this study was to compare different intervention strategies against clinical intramammary infections (IMI). We conducted a simulation study representing a Danish dairy cattle herd and ten different intervention strategies against clinical IMI. As standard intervention, a 3-day intramammary treatment for all clinical cases was taken. Two strategies reflected the use of more antibiotics, six strategies reflected cow-specific treatment or culling decisions, and one strategy reflected a herd with better hygiene. For these strategies, we investigated costs and effectiveness of culling as an IMI intervention and the influence of the transmission level on the net income. Our results showed that nearly all strategies could reduce the number of IMI cases compared to the standard intervention. This happened either at the cost of an increased antibiotic usage or an increased number of cows culled in relation to IMI. However, substantial economic benefits could only be seen if the transmission level was reduced, i.e. in the herd with better hygiene. In this case, both antibiotic treatments and number of culled cows were reduced. The potential economic gain from a reduced transmission level could be used to improve hygiene without resulting in an overall loss. Therefore, it should be recommended to reduce IMI transmission in a dairy herd to a low level through hygiene measures, maybe in combination with a cow-specific clinical intervention approach.

KEY WORDS: dairy cattle, clinical mastitis, treatment, culling

INTRODUCTION

Mastitis, or intramammary infection (IMI), is frequently found on dairy farms and causes considerable economic losses (e.g., Halasa et al., 2007) as well as impairs animal welfare (Broom, 1991; von Keyserlingk et al., 2009). IMI also contribute a major part to the use of antibiotics in dairy cattle in Denmark (DANMAP, 2016, p. 24; EMA and EFSA, 2017, p. 29).

Costs for clinical IMI can be divided into costs associated with treatment, costs from increased mortality through culling, i.e. for replacement of culled animals, and indirect costs from production losses (e.g., Halasa et al., 2007), and were previously investigated in various studies (e.g., Bar et al., 2008). These studies rely on a modelling approach, where a farm with IMI is simulated and the arising costs are calculated. Some focus on modelling occurrence or transmission of IMI causing pathogens (e.g., Allore et al., 1998; Hagnestam-Nielsen and Østergaard, 2009; Halasa et al., 2009a; Østergaard et al., 2005). Others investigate intervention (e.g., Halasa, 2012; Steeneveld et al., 2011) or replacement strategies (e.g., Cha et al., 2014). Many of these studies also consider the IMI causing pathogen and are thus pathogen-specific (e.g., Allore et al., 1998; Halasa et al., 2009a; Østergaard et al., 2005), as the effects of an IMI, e.g. milk production losses, may depend on the causative pathogen (Gröhn et al., 2004; Hertl et al., 2014).

Usually, investigated intervention strategies for clinical cases consist of antibiotic treatment, mostly intramammary. However, in the light of rising consumer awareness regarding antibiotic usage in food animals and its connection with antimicrobial resistance (Ruegg, 2003), it may be sensible to search for alternative intervention strategies. For instance, culling cows with clinical mastitis could be a valid alternative. In contrast to antibiotic treatment, however, culling of infected animals is rarely considered as an intervention strategy for IMI, but rather as a possible consequence of clinical IMI (Halasa and Hogeveen, 2018). It has previously been studied in the context of optimal replacement decisions (e.g., Cha et al., 2014; Heikkilä et al., 2012), leading to only slightly earlier optimal replacement time for cows with clinical IMI and thus recommending treatment over culling in most cases. These studies evaluated optimal replacement time in terms of economics, comparing the results for cows with and without IMI. Yet, specific culling strategies for clinical IMI have to our knowledge not been the focus of a study.

Besides antibiotic treatment or culling of cows with clinical mastitis, it may also be economically beneficial to lower transmission of IMI causing pathogens, for instance by improving hygiene or biosecurity. However, if pathogen transmission is contagious, the number of new cases depends on the number of infected

animals (Halasa et al., 2009a). Lowering the transmission of IMI causing pathogens may therefore lead to a long-term indirect cost reduction due to a decrease in the number of new cases (Steeneveld et al., 2011). Consequently, the long-term effects of lowering transmission have to be evaluated with a model that includes transmission dynamics as in Halasa's study (2012).

The aim of the current study was to evaluate different IMI intervention strategies, using three distinct approaches (using more antibiotics, reactive culling, and reducing the transmission rate by, e.g., improving herd hygiene or increasing biosecurity). For this purpose, cow- and pathogen-specific transmission of IMI was modelled for a Danish dairy cattle herd with 200 dairy cows at different transmission levels. Subsequently, strategies with cow-specific antibiotic treatment of clinical cases, cow-specific culling of cows with clinical IMI, and improving hygiene at the farm were compared for farm economics and epidemiological parameters, including the number of clinical IMI cases, culled cows, and antibiotic doses (treatment days).

MATERIALS AND METHODS

Herd and Transmission Model

HERD MODEL. The model used in this study is the MiCull (Mastitis-iCull) model, Version 2.0. The original MiCull model Version 1.0 (Gussmann et al., 2018b) was used in Kirkeby et al. (2017) and differs only in the possible interventions for clinical IMI, which are new in Version 2.0, as explained below. The model and all simulations were programmed and run in the statistical computing software R version 3.2.2 "Fire Safety" (R Core Team, 2016). Figures were made using the packages ggplot2 (Wickham, 2009).

In the model, a dairy herd with 200 dairy cows is simulated in single-day time steps (Kirkeby et al., 2016). The cows are distributed in five compartments (calves, heifers, lactating cows, dry cows, calving area). After a stochastic number of days, each cow moves on to the next compartment if it was not culled. Feeding depends on which compartment a cow is in and, for

lactating cows, takes the produced milk into account. Lactation (milk, protein, and fat) and somatic cell count (SCC) curves are cow-specific (Græsbøll et al., 2016) and adjusted for IMI effects, i.e. increased SCC (Schepers et al., 1997; Wilson et al., 1997) and decreased milk yield (Gröhn et al., 2004; Hortet et al., 1999). Milk from clinical cows is withdrawn during antibiotic treatment and for six days afterwards. Once a month, milk yield and SCC are recorded. The future average milk production (FAP) of a cow was estimated (Græsbøll et al., 2017).

TRANSMISSION FRAMEWORK. The model currently includes five pathogen strains that can cause IMI, a contagious *Staphylococcus aureus*, environmental *Escherichia coli*, both a contagious and an environmental *Streptococcus uberis*, and *Streptococcus agalactiae* with both contagious and environmental elements combined. The IMI transmission module was adapted and extended from Halasa et al. (2009a, 2010). Heifers are modelled separately from the lactating herd until they calve, where they have a certain probability to enter their first lactation infected. For new infections in lactating cows, the infection probability for every non-infected quarter is calculated. This probability depends on the active pathogen strains, on the number of infected quarters for contagious strains, and on the cow's susceptibility. The susceptibility of a cow takes risk factors as parity and previous IMI into account (Zadoks et al., 2001) and adjusts susceptibility relative to previously uninfected primiparous cows. Every new infection has a pathogen-specific probability to immediately appear as a clinical case, otherwise it will be subclinical (Halasa et al., 2009a). Furthermore, previously infected quarters have a certain pathogen-specific probability for spontaneous recovery from the subclinical state or for flare up from subclinical to clinical. Clinical quarters are treated with a 3-day antibiotic intramammary treatment, after which the clinical quarter will return to either susceptible or subclinical, depending on the cow-specific recovery probability (Steeneveld et al., 2011).

If a cow had a clinical IMI during the lactation or a high SCC (200,000 or higher) at one of the last three monthly recordings, a pooled milk sample will be sent for testing by polymerase chain reaction (PCR, sensitivity and specificity are given in Table III.1) before dry off. Cows with a positive PCR test result receive

Parameter	Description	Value	Reference
Transmission rate	Rate for susceptible quarters to enter infected state	0.0009	Fitted values
Probability of clinical state	Probability for a quarter to enter clinical state upon infection	0.17	See Table III.S1
Flare up probability	Probability for a subclinical quarter to become clinical	0.0081	See Table III.S1
Spontaneous recovery probability	Probability for a subclinical quarter to become susceptible (without treatment)	0.0064	See Table III.S1
Recovery probability	Probability for a clinical quarter to become susceptible after treatment	0.4	See Table III.S1
Test sensitivity	Test sensitivity for PCR (used at dry off)	0.908	Mahmmod et al. (2013)
Test specificity	Test specificity for PCR (used at dry off)	0.988	Mahmmod et al. (2013)
Probability to identify pathogen	Probability to identify causative pathogen by PCR	0.85	Taponen et al. (2009)

Table III.1: Model parameters for the main causative pathogens *S. aureus* during lactation

antibiotic dry cow treatment, while cows with a negative result will be dried off without dry cow treatment. New infections can occur during the first and last week of the dry period, but infection probabilities do not depend on the number of infected quarters and are lower for cows that received dry cow treatment (Halasa et al., 2009b). Similarly, subclinical cases can only flare up to clinical cases in the first and last week of the dry period and only for cows without dry cow treatment. Cows that become clinical in the first week, will also receive dry cow treatment. Spontaneous recovery can occur during the whole dry period (Halasa et al., 2010).

CULLING. Once a week, if the number of lactating and dry cows exceeds the target count of 200 dairy animals, cows are evaluated for culling (Kirkeby et al., 2016). All cows get weighted flags for low milk yield, parity, reproduction status, high SCC, and previous cases of clinical IMI, and the cows with the highest weights are culled. There is also a certain probability that some cows have to be culled for other reasons, e.g., lameness (Kirkeby et al., 2016). This involuntary culling takes precedence over voluntary culling. Costs for culling are around €1,000 (price of a new heifer), not including the income from slaughter. However, for every culled cow, the mean slaughter value is deducted, resulting in less than €1,000 cost for every culled cow.

Intervention Strategies for Clinical IMI

A Danish dairy cattle herd with a default yearly median cumulative clinical incidence of about 21 % on cow level, mostly caused by *S. aureus*, was simulated for this study.

INDIFFERENT TREATMENT FOR ALL COWS (BASIC3, BASIC5). The default intervention in the model for clinical IMI is a 3-day intramammary treatment (Basic3). A second strategy consists of an extended intramammary treatment for five days (Basic5). In all following strategies, treatment will always be 3-day intramammary treatment, unless specified otherwise.

LONGER TREATMENT FOR HIGH PRODUCING COWS (LONGER). As described above, the FAP was used to determine cows in the top 25 % of expected future milk production. In this strategy, these cows are treated for five instead of the usual three days.

CULLING OF REPEATED CLINICAL IMI CASES (REPEATED). For this strategy, cows showing a clinical IMI for the second time in their current lactation are culled instead of treated. Testing Before Treatment (Before50 and Before75). Here, clinical cases are not immediately treated. Instead, a milk sample of the new clinical quarters will be sent for testing by PCR with 85 % probability to identify the causative pathogen, as described by Taponen et al. (2009). Test results will return one day later, and are used to calculate the expected recovery probability, based on the causative pathogen, history of IMI, parity, days in milk (DIM) of the cow, and SCC at the last monthly milk recording (Steeneveld et al., 2011). If the pathogen could not be identified, a mean base cure probability is used instead of the cure probability for the actual causative pathogen. The estimated recovery probability is used to decide whether a cow will be culled (recovery probability below 50 % (Before50) or 75 % (Before75)) or treated.

CULLING WITH EXCEPTIONS (NOTCULLTOP AND NOTCULLPREGNANT).
With the Before75 strategy, the farmer may also decide not to cull a certain group of cows, e.g., cows in the top 25 % according to

the FAP (notCullTop), or cows that are more than approximately 4 months pregnant (notCullPregnant). These cows are always treated without sending a milk sample for testing.

TESTING AFTER TREATMENT (AFTER). Similarly to testing before treatment, in this strategy quarters are tested by bacterial culture seven days after treatment ended. Test sensitivity and specificity are respectively 0.523 and 0.895 (Mahmmod et al., 2013). If the test result is positive when it returns one day later, the cow will be culled.

BETTER HYGIENE (HYGIENE). Strictly spoken, this strategy did not represent a clinical IMI intervention, but rather a similar herd with a better hygiene. This was simulated by using a lower transmission rate, due to better milking and general hygiene, resulting in a low cumulative clinical incidence of about 8 % (Hygiene). Clinical IMI intervention in this herd consisted of 3-day intramammary treatment.

Simulations and Model Output

For each of the ten described strategies, we ran 500 iterations of a five year period with an additional five years of burn-in time, to insure stable results describing the effect of the strategies rather than the initial parameter values (transmission parameters are given in Table III.1).

Model output was collected in the simulated five year period and included economics in the form of income from milk (which is calculated from fat and protein price, a milk handling fee, and a penalty or bonus depending on the bulk tank SCC), costs related to IMI (testing, treatment including opportunity costs, culling, dry cow treatment), and other costs (culling with a high SCC or history of IMI, feed), with prices given in Table III.2. The mentioned costs were subtracted from the income from milk to calculate a mean (over five years) yearly income for the farm, from which additional expenses (e.g., costs for other diseases, or running costs) have yet to be deducted. The output also included epidemiological parameters, e.g., the number of clinical cases (quarters entering clinical state from susceptible

	Price	Reference
1 kg protein	5.8132	www.arla.dk, September 2017
1 kg fat	4.1519	www.arla.dk, September 2017
Handling of 1 kg milk	−0.01343	www.arla.dk, September 2017
Slaughter value per cow	483	Kudahl et al. (2007)
Feeding		
Per calf per day	−0.0026	Kirkeby et al. (2016)
Per heifer/dry cow per day	−0.9311	Kirkeby et al. (2016)
Per energy corrected milk	−0.1947	Kirkeby et al. (2016)
Treatment (per day)	−11.10	Michael Farre (SEGES, Aarhus, Denmark, personal communication)
Opportunity cost (per case per day)	−6.66	Halasa et al. (2009a); Michael Farre (SEGES, Aarhus, Denmark, personal communication)
Dry cow treatment	−9.60	Michael Farre (SEGES, Aarhus, Denmark, personal communication)
Bacterial culture	−12	Michael Farre (SEGES, Aarhus, Denmark, personal communication)
PCR	−13.3	Michael Farre (SEGES, Aarhus, Denmark, personal communication)

Table III.2: Prices in EUR used in the model to calculate income (positive values) and costs (negative values)

or subclinical state), the number of subclinical cases (quarters entering subclinical state from susceptible or clinical state), the number of culled cows (due to IMI intervention, or with a high SCC or history of IMI) and the number of treatment days (a 3-day treatment of a clinical quarter equals three treatment days) over the simulated five year period. The numbers are rounded to integers, or in the case of percentages to one decimal.

Sensitivity analyses

Sensitivity analyses were performed for test characteristics, i.e. probability to identify the pathogen according to Taponen et al. (2009), or test sensitivity and specificity according to Cederlöf et al. (2012); base cure probability after treatment; fat and protein prices for 2017 in Denmark (www.arla.dk/om-arla/ejere/arlapris/2017); prices for culling; as well as transmission rates and causative pathogens, reflecting different herds (Table III.3).

RESULTS

All results for the mentioned strategies can be found in Table III.4. The numbers presented are rounded median values (with the 5th and 95th percentiles) of the annual average over the five

Sensitivity analysis	Values				
Test characteristics					
Sensitivity / specificity of bacterial culture	0.52/0.9*	0.78/0.97	0.83/0.97	0.88/0.94	0.94/0.9
Probability to identify a pathogen by PCR	0.9	0.85*	0.8	0.75	0.5
Base cure probability	0.2	0.4*	0.6	0.7	
Milk price					
Price for 1 kg protein / 1 kg fat in EUR	5.25/3.75	5.51/3.94	5.66/4.04	5.81/4.15*	5.92/4.23
Additional costs for culling in EUR	0*	250	500	1,000	
Transmission rates per quarter per day					
Low incidence herd					
emphS. aureus		0.00004			
emphS. uberis (contagious)		0.0002*			
emphS. uberis (environmental)		0.000002*			
High incidence herd (*S. aureus*)		0.0018			
Medium incidence herd (contagious *S. uberis*)		0.0047			
Medium incidence herd (environmental *S. uberis*)		0.00009			

Table III.3: Values used in the sensitivity analyses, default values (Table III.1 and III.2) are marked by *

simulated years. Clinical and subclinical cases are always quarter cases.

In the basic strategy (Basic3), there were 42 clinical cases per year in median, ranging from 33 to 51 cases for the 5th and 95th percentiles, respectively (Table III.4). These cases led to a median 123 treatment days per year. There were 136 subclinical cases in median, and in median 16 cows were culled with a high SCC or a history of IMI. The median yearly income in the Basic3 strategy was €187,666 (Table III.4).

Comparable numbers for clinical cases and culled cows could be observed when the high producing cows were treated for five days instead of three (Longer), but with a higher median and variance in treatment days. The median number of subclinical cases was lower, with a higher variance skewed to the left (Table III.4). If all cows were treated for five days (Basic5), the median number of clinical cases and of subclinical cases was lower and the median number of treatment days increased. The number of culled cows seemed slightly lower (Table III.4). In these strategies, the median yearly income remained similar (Table III.4).

In the strategy Repeated, where cows with repeated clinical IMI cases were culled, the number of clinical cases resembled the number of clinical cases in Basic5, though with less treatment days and more culled cows, which also included cows culled as IMI intervention (Table III.4). In all other strategies that included reactive culling, there were less clinical cases, with the smallest median of 29 cases in strategy Before75. The numbers

Strategy	Net income		Clinical IMI cases		Subclinical IMI cases		Treatment days		Culled cows	
Basic3	187,666	(173,363; 202,147)	42	(33; 51)	136	(121; 161)	123	(95; 151)	16	(12; 20)
Basic5	190,014	(175,823; 205,741)	37	(19; 46)	123	(86; 145)	179	(93; 220)	15	(11; 18)
Longer	188,307	(175,953; 203,414)	41	(27; 51)	133	(109; 157)	129	(81; 162)	16	(12; 19)
Repeated	191,280	(179,588; 205,338)	38	(21; 46)	125	(90; 145)	86	(53; 106)	20	(14; 25)
After	191,699	(179,990; 207,164)	35	(15; 43)	129	(80; 147)	101	(46; 125)	22	(13; 27)
Before50	196,995	(181,427; 211,492)	30	(15; 38)	111	(75; 130)	47	(36; 64)	24	(10; 29)
Before75	197,576	(184,745; 213,907)	29	(13; 35)	106	(71; 120)	7	(4; 11)	32	(18; 38)
notCullTop	196,704	(182,197; 213,318)	32	(14; 39)	113	(72; 127)	32	(12; 43)	29	(16; 34)
notCullPregnant	195,600	(184,007; 210,633)	31	(14; 37)	112	(74; 123)	24	(12; 32)	30	(17; 35)
Hygiene	205,326	(193,513; 217,172)	16	(13; 24)	80	(72; 102)	47	(37; 71)	11	(8; 14)

Table III.4: Median model output (with fifth and ninety-fifth percentiles) of 500 iterations for a herd with 200 dairy cows, simulated over 5 years: Yearly income in € (income from milk minus costs related to IMI and for feeding), yearly number of clinical IMI cases, yearly number of subclinical IMI cases, yearly number of treatment days, and yearly number of culled cows (due to IMI intervention, or with a high SCC or history of IMI). Yearly numbers are means over five simulated years. Numbers are rounded.

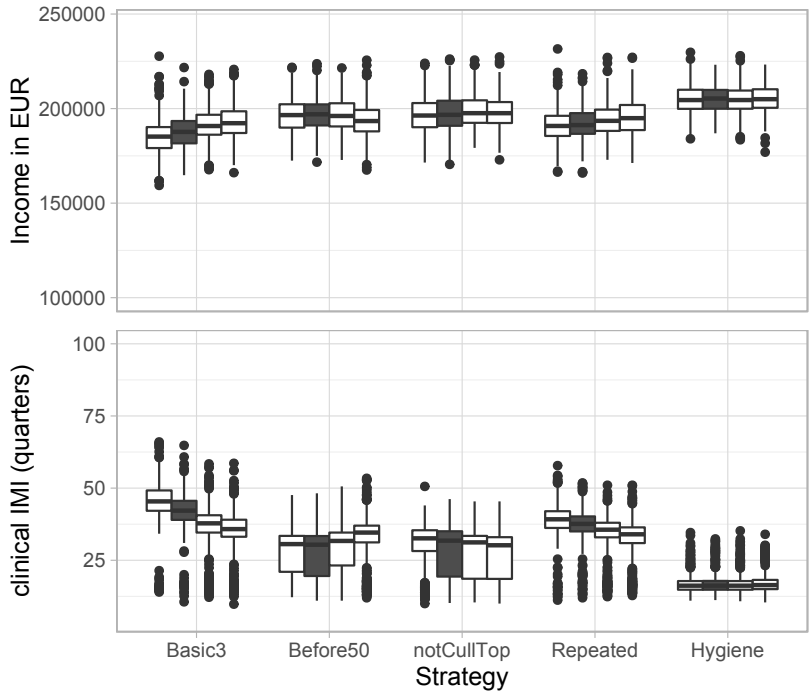

Figure III.1: Results of the sensitivity analysis for the base cure rates in selected strategies (increasing cure rates from left to right, Table III.3). Box plots show yearly income (income from milk minus costs for IMI intervention, dry cow treatment, feed, and culling) and yearly cumulative clinical IMI incidence for 500 iterations. Gray boxes show results with default values. For each iteration, means over the simulated five year period were taken.

of subclinical cases in strategies Repeated and After were higher than in Basic5, though lower than in Basic3 and Longer. All other strategies including culling showed fewer subclinical cases, with the smallest median of 106 in strategy Before75. All culling strategies had a larger number of culled cows than the strategies without culling as an intervention, up to a median of 32 cows culled per year in strategy Before75. In return, the median number of treatment days was lower, with a minimum of 7 treatment days in median in strategy Before75. Generally, the numbers of culled cows and treatment days were reversed: more treatment days corresponded to less culled cows. An exception was the

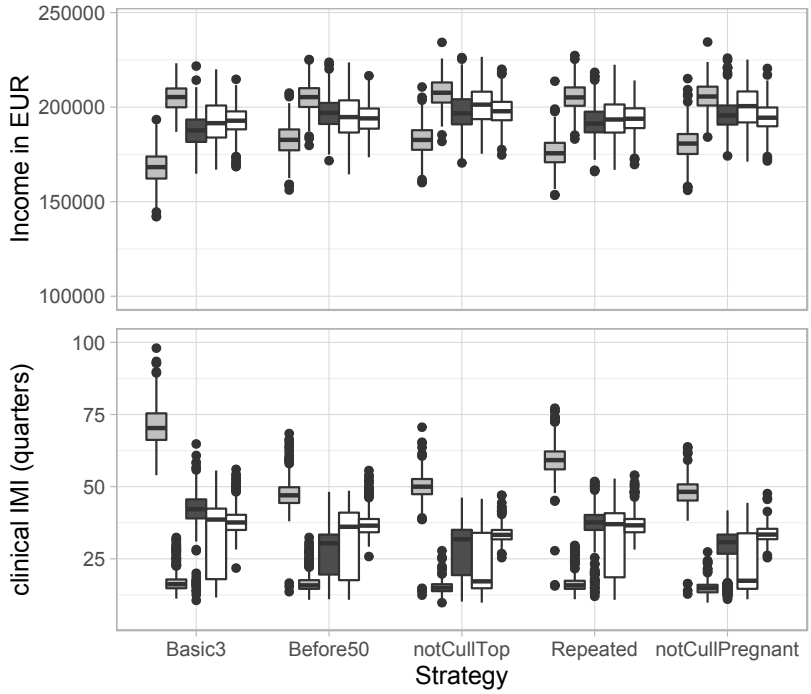

Figure III.2: Results of the sensitivity analysis for transmission rate (light gray, left: low transmission rates, right: high transmission rate *S. aureus*, Table III.3) and main causative pathogens (white, left: contagious *S. uberis*, right: environmental *S. uberis*, Table III.3). Box plots show yearly income (income from milk minus costs for IMI intervention, dry cow treatment, feed, and culling) and yearly cumulative clinical IMI incidence for 500 iterations. Dark gray boxes show results with default values (Table III.1). The leftmost box for strategy Basic3 is equivalent to strategy Hygiene. For each iteration, means over the simulated five year period were taken.

strategy After, which had both more treatment days and more culled cows than the strategy Repeated (Table III.4). The median yearly income in the strategies Repeated and After was around €191,500. In the strategies Before50, Before75, notCullPregnant, and notCullTop it varied from €195,600 (notCullPregnant) to €197,576 (Before75).

The low transmission herd (Hygiene) yielded a higher median yearly income than any intervention strategy and the lowest median number of clinical cases, subclinical cases and culled cows. The number of treatment days was comparable to Before50, with half the number of cows culled in relation to IMI.

Sensitivity analysis (see Table III.3 for input values) on test characteristics showed that varying the probability to identify the pathogen by PCR before treatment did not lead to large changes in the results. When increasing sensitivity (and thus decreasing specificity) for bacterial culture in strategy After, the income did not change much, while the number of clinical IMI cases and treatment days per year decreased (Figure III.S1).

An increased base cure probability after treatment led to increased income and decreased number of clinical IMI, treatment days and culled cows in all strategies, though the effect was less visible in strategies where cows are tested and culled before treatment. In strategy Before50, the number of clinical IMI and treatment days increased with increasing base cure probability (Figure III.1). With low transmission rates (Hygiene), the outcome was not sensitive to the base cure probability.

Sensitivity analysis for the fat and protein prices according to Danish prices in 2017 showed that the yearly income is highly dependent on the milk price, with medians ranging from €120,597 to €200,096 in the Basic3 strategy. The other strategies showed a similar pattern in the relation between milk price and yearly income (Figure III.S2).

When culling was more expensive, the yearly income was lower. In strategies with more culled cows, this effect was more pronounced (Figure III.S3). This led to greater discrepancies in the yearly income among the different intervention strategies.

Sensitivity analyses for transmission rates led to varying cumulative clinical incidences. When a herd with a default median cumulative clinical incidence of around 19 % was modelled,

where most cases were caused by an environmental *S. uberis* strain, results were mostly similar (Figure III.2). If most cases where caused by a contagious *S. uberis* strain, differences were more noticeable (Figure III.2). For one, the variation in the results was higher. In the strategies Before75 (results not shown), notCullPregnant, and notCullTop, the median number of clinical cases and culled cows was lower, though the range of results was comparable. Similar to the environmental strain, the contagious *S. uberis* strain led to a higher number of clinical cases and a lower median yearly income in the strategy Before50. In the other strategies, the median yearly income was higher for both *S. uberis* strains.

Results for other strategies than 3-day intramammary treatment in a herd with a lower (8 %) median cumulative clinical incidence can be seen in Figure III.2. In a herd with a low number of clinical cases, the yearly income was higher and the differences between the different intervention strategies seemed smaller. Contrarily, in a herd with a higher (35 %) median cumulative clinical incidence, where most cases were caused by *S. aureus*, the income was lower and the differences between strategies were bigger. There, the cumulative clinical incidence could be reduced down to a median of 22 % through intervention strategy Before75 (results not shown), which also decreased the number of treatment days drastically. However, 23 % of the cows were culled with reasons related to IMI (results not shown).

DISCUSSION

The objective of this study was to evaluate different intervention strategies against clinical IMI. In the study, ten strategies were presented. In the default strategy (Basic3), there was no reactive culling and all clinical cases were treated intramammary for three days. Two strategies reflected interventions with increased antibiotics usage (Basic5, Longer). Six interventions explicitly included culling as an intervention against IMI, where a cow-specific decision to cull or treat a cow with clinical IMI was taken. Reactive culling could happen instead of treatment (Before50, Before75, notCullTop, notCullPregnant, Repeated) or for not

cured cows (After). The last strategy reflected a herd with better hygiene (Hygiene).

Although various previous studies have investigated IMI intervention and culling in relation to clinical IMI (e.g., Cha et al., 2014; Halasa, 2012; Heikkilä et al., 2012), culling as a strategy against clinical IMI, as presented in the current study, has rarely been considered as a reactive measure (Halasa and Hogeveen, 2018). Furthermore, in other IMI intervention studies, IMI transmission was seldom taken into account. In the present study, we modelled both IMI transmission and interventions, allowing an evaluation of different intervention strategies' long-term effects. Another new aspect of the current study was that both economics and epidemiological consequences are presented as output. Farmers are not solely interested in farm economics, but also in other perceived benefits (Jansen and Lam, 2012; Valeeva et al., 2007). Clinical IMI cases are perceived as both cost and time intensive (Jansen et al., 2009), thus presenting the number of clinical cases in addition to the economics could provide farmers different incentives to adopt a new strategy. The number of treatment days and cows culled in relation to IMI are also shown to give an estimate for respectively antibiotic usage and longevity, which may also affect the choice of an intervention strategy.

Our results showed that economically, i.e. in their yearly income, all strategies seemed more successful than indifferent 3-day intramammary treatment for all clinical cases (Basic3). Using more antibiotics (Basic5, Longer) led to only a small increase in yearly income, while the increase was higher in intervention strategies with reactive culling. Here, testing newly clinical quarters and subsequent cow-specific treatment or culling decisions (Before50, Before75, notCullTop, notCullPregnant) led to a higher income than testing a week after treatment (After) or culling cows with repeated cases (Repeated). These results are not consistent with an earlier study that found cow-specific treatment not to be economically beneficial (Steeneveld et al., 2011). Nevertheless, this study did not model IMI transmission. By including transmission, the reduced number of IMI cases due to an intervention strategy could be taken into account, which in turn can explain the higher yearly income, consistent with the findings of Halasa (2012).

Increasing the sensitivity of bacterial culture resulted in fewer cases (Figure III.S1). This result is concordant with van den Borne et al. (2010a), who showed that test sensitivity to detect subclinical IMI had substantial impact on the cost-effectiveness of control strategies for subclinical IMI. The results were also sensitive to the cure (Figure III.1) and transmission rates (Figure III.2), which is consistent with studies by Halasa (2012) and Down et al. (2013). Unfortunately, there exist only a small number of studies estimating the transmission rates of few IMI causing pathogens. Further studies are needed, also with focus on assessing the impact of control strategies on the transmission of IMI causing pathogens. This would allow a more precise assessment of the cost-effectiveness of IMI control in dairy herds.

Sensitivity analysis for the cost of culling showed that with increasing culling costs, the income decreased (Figure III.S3). The opposite was the case for increased milk prices (Figure III.S2). Increased culling costs or reduced milk prices may therefore have a substantial effect on the cost-effectiveness of an intervention strategy. Hence, these factors must be taken into account when deciding on which intervention strategy to adopt, especially if reactive culling is being considered. It is important to mention that we had fixed prices over time. We acknowledge that this is unrealistic, but it is not only difficult to predict changes in prices; fixing a price also removes extra noise that would make it more difficult to compare different strategies.

Together, our results showed that compared to an indifferent 3-day intramammary treatment for all clinical quarters, there were three possibilities to improve a herd's IMI situation, i.e. to reduce the number of not only clinical but also subclinical quarter cases. The first was to increase the usage of antimicrobials for treatment of clinical IMI cases (Basic5). If this is not desired, an alternative was to cull reactively (After, Repeated, Before50, Before75, notCullTop, notCullPregnant). In this case, antibiotic treatment could be greatly reduced, but the number of cows culled in relation to IMI increased. A decision between these two options could be seen as a decision between an increased risk for antimicrobial resistance (more antibiotics) or decreased longevity (more culling). From an economic point of view, culling seemed to be the better choice in the studied herd with a medium cumu-

lative clinical incidence. However, for a high cumulative clinical incidence, this distinction was not as clear and depended on the individual culling strategies (Figure III.2), while it was nearly indiscernible when the cumulative clinical incidence was low (Figure III.2). The third option, reducing the transmission level in the herd (Hygiene), was the only possibility to decrease IMI cases, antibiotic treatment, and IMI related culling at the same time. In fact, the strategy Hygiene and the sensitivity analysis on the transmission rate of the main causative pathogen showed that solely adopting an intervention strategy for clinical IMI was not enough to reach a stable low incidence in a herd that was comparable with a low transmission due to good hygiene. Unfortunately, there are only very few studies investigating the effect of hygienic measures. Lam et al. (1996) investigated the effect of postmilking teat disinfection and found that it reduced the transmission rate noticeably. Huijps et al. (2010) estimated the costs and effects of various separate hygienic measures, however, not in terms of transmission rates. Due to the lack of studies investigating the effect of a comprehensive hygienic strategy on the transmission rate of IMI pathogens within a herd, we did not include the costs arising from such a comprehensive hygienic strategy in this study. This makes it difficult to properly assess the cost-effectiveness of the strategy Hygiene. Nevertheless, there was a considerable profit in reducing the transmission rate in Hygiene, though it remains to be shown if the economic gain would be enough to improve herd hygiene enough to reach the assumed lower transmission rate. Even so, from an epidemiological point of view, improving hygiene led to better results than a higher use of antibiotics or more culling. However, the choice of which approach to take should also depend on the specific herd and situation, as different intervention strategies may be preferable depending on the main causative pathogen strain. The decision has to be taken by the farmer, who has to decide which option fits best to his/her beliefs or ideas for the respective herd.

In this study, we do not consider non-antibiotic treatment for IMI. To investigate this possibility, the model would also have to include a distinction between mild, moderate, or severe clinical IMI cases, as non-antibiotic treatments are mainly considered for

mild or moderate clinical IMI (e.g., McDougall et al., 2016, 2009). This could be considered in future studies.

Also, some of the strategies presented in this paper involved strict culling rules, that farmers may not want to adhere to. For that reason, we considered strategies where groups of animals were excluded from culling, e.g., cows that were more than about four months pregnant (notCullPregnant) or high-producing cows (notCullTop). The latter strategy particularly considers an earlier study on determinants for antimicrobial treatment, where in some Danish herds, high producing cows were more likely to be treated (Gussmann et al., 2018a). We also simulated other strategies, e.g., higher chances for heifers to be treated. However, results showed to be quite similar, so they were not presented here. Nevertheless, Vaarst et al. (2006) found that there were farmers willing to adopt a culling strategy that matched their goal for the herd, so including some form of reactive culling in IMI intervention strategies is not unreasonable. Still, this kind of culling affects the usual culling procedure, especially considering that we modelled a closed herd, where replacement heifers had to be available on-herd. This may lead to problems when a larger number of animals have to be culled due to other, non-voluntary reasons, e.g., other diseases. However, in this study we concentrated on IMI interventions and their economic and epidemiological effects, leaving future studies to further investigate culling dynamics as a whole.

CONCLUSIONS

We showed that cow-specific intervention strategies against IMI including reactive culling can be economically beneficial in the long term, even more so than a strategy with increased antibiotic treatment. The increased income from milk together with the reduced number of IMI cases compensated for the extra intervention costs, though economic benefits also depended on the transmission level in the herd. All strategies except one could also reduce the number of clinical cases compared to indifferent 3-day intramammary treatment. This happened either at the cost of an increased antibiotic usage, or at the cost of the number

of cows culled in relation to IMI. Therefore, the farmer has to choose the right balance between treatment and culling for a specific herd, given his/her goals for antimicrobial usage and longevity, as well as the main causative pathogen in the herd. With this study, we have shown that cow-specific treatment or culling decisions will in most cases reduce the incidence of clinical IMI, while increasing the farm's income in the long term. However, the best option to reach a stable low number of IMI cases would be to reduce IMI transmission. Unfortunately, data is missing to assess cost-effectiveness of this strategy.

ACKNOWLEDGEMENTS

The authors thank Tine van Werven for her input in discussing interventions against clinical mastitis and her suggestions for additional intervention strategies. This study is part of the STOPMAST project, funded by the Danish Milk Levy Foundation (Mælkeafgiftsfonden), and part of the EMCo-Mast project, funded by the Green Development and Demonstration Program (GUDP) under the Danish Directorate for Food, Fisheries and Agriculture, grant no. 34009-15-0918.

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SUPPLEMENTARY MATERIAL

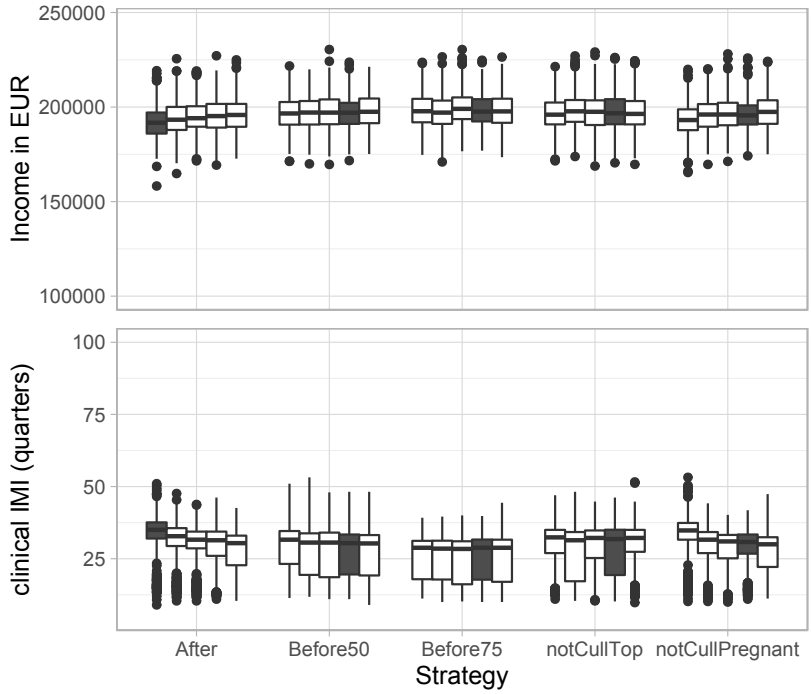

Figure III.S1: Results of the sensitivity analysis for sensitivity and specificity of bacterial culture (After; increasing sensitivity from left to right, Table III.3) and probability to identify the causative pathogen (Before50, Before75, notCullTop, notCullPregnant; increasing probability from left to right, Table III.3) in selected strategies. Box plots show yearly income (income from milk minus costs for IMI intervention, dry cow treatment, feed, and culling) and yearly cumulative clinical IMI incidence for 500 iterations. Gray boxes show results with default values. For each iteration, means over the simulated five year period were taken.

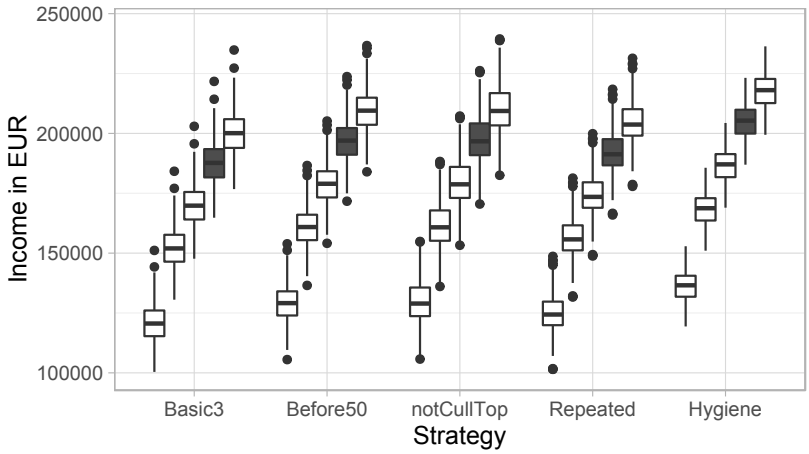

Figure III.S2: Results of the sensitivity analysis for milk prices (increasing price from left to right, Table III.3). Box plots show yearly income (income from milk minus costs for IMI intervention, dry cow treatment, feed, and culling) and yearly cumulative clinical IMI incidence for 500 iterations. Gray boxes show results with default values. For each iteration, means over the simulated five year period were taken.

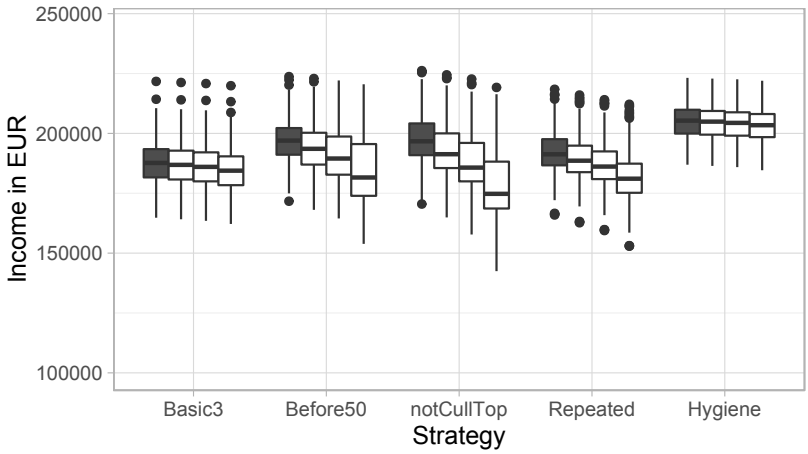

Figure III.S3: Results of the sensitivity analysis for culling costs (increasing price from left to right, Table III.3). Box plots show yearly income (income from milk minus costs for IMI intervention, dry cow treatment, feed, and culling) and yearly cumulative clinical IMI incidence for 500 iterations. Gray boxes show results with default values. For each iteration, means over the simulated five year period were taken.

Parameter	Description	Value (lactation)	Value (dry period)	Reference (value lactation)
Transmission rate	Rate for susceptible quarters to enter infected state			
	S. aureus	0.00004	0.0009	Fitted value
	S. agalactiae	0.0007	0.0001	Fitted value
	S. uberis (contagious)	0.0002	0.00001	Fitted value
	S. uberis (environmental)	0.000002	0.0000001	Fitted value
	E. coli	0.000001	0.000001	Barkema et al. (1998)
Probability of clinical state	Probability for a quarter to enter clinical state upon infection			
	S. aureus	0.17	0.1	Swinkels et al. (2005a)
	S. agalactiae	0.01	0.1	Barkema et al. (1998) and Swinkels et al. (2005a)
	S. uberis (contagious)	0.32	0.1	Zadoks et al. (2003)
	S. uberis (environmental)	0.32	0.1	Zadoks et al. (2003)
	E. coli	0.85	0.1	Hogan and Smith (2003)
Flare up probability	Probability for a subclinical quarter to become clinical			
	S. aureus	0.0081	0.006	Swinkels et al. (2005a)
	S. agalactiae	0.0005	0.0005	Barkema et al. (1998) and Swinkels et al. (2005a)
	S. uberis (contagious)	0.0068	0.004	Swinkels et al. (2005b)
	S. uberis (environmental)	0.0068	0.004	Swinkels et al. (2005b)
	E. coli	0.0035	0.0035	Döpfer et al. (1999)
Spontaneous recovery probability	Probability for a subclinical quarter to become susceptible (without treatment)			
	S. aureus	0.0064	0.0079	van den Borne et al. (2010b)
	S. agalactiae	0.0023	0.0086	Leelahapongsathon et al. (2016)
	S. uberis (contagious)	0.0143	0.0086	van den Borne et al. (2010b)
	S. uberis (environmental)	0.0143	0.0086	van den Borne et al. (2010b)
	E. coli	0.0221	0.0221	van den Borne et al. (2010b)
Recovery probability	Probability for a clinical quarter to become susceptible after treatment or dry cow treatment			
	S. aureus	0.4	0.77	Steeneveld et al. (2011)
	S. agalactiae	0.7	0.89	Steeneveld et al. (2011)
	S. uberis (contagious)	0.7	0.89	Steeneveld et al. (2011)
	S. uberis (environmental)	0.7	0.89	Steeneveld et al. (2011)
	E. coli	0.8	0.9	Steeneveld et al. (2011)

Table III.S1: Model parameters for all secondary pathogens during lactation and dry off, references are given for values during lactation. Dry period values are taken from Halasa et al. (2010).

4.5 MANUSCRIPT IV

ECONOMIC AND EPIDEMIOLOGICAL IMPACT OF DIFFERENT INTERVENTION STRATEGIES AGAINST SUBCLINICAL AND CLINICAL MASTITIS

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Submitted to *Journal of Dairy Science*

ABSTRACT

The objective of this study was to evaluate and compare different combinations of intervention strategies against contagious or opportunistic subclinical and clinical intramammary infections (IMI). We simulated two different Danish dairy cattle herds with ten different interventions strategies, including three baseline strategies without subclinical interventions. In one herd, the main causative pathogen of IMI was *Staphylococcus (S.) aureus*. In the other herd, *Streptococcus (St.) agalactiae* was the main causative agent. For both herds, we investigated costs and effectiveness of all ten intervention strategies. Intervention strategies consisted of measures against clinical and subclinical IMI, with baselines given by purely clinical intervention strategies. Our results showed that strategies including subclinical interventions were more cost-effective than the respective baseline strategies. Increase in income and reduction of IMI cases came at the cost of an increased antibiotic usage and an increased culling rate in relation to IMI. However, there were differences between the herds. In the *St. agalactiae* herd, the clinical intervention strategy did not seem to have a big impact on income and number of cases. However, intervention strategies which included cow-specific clinical interventions led to a higher income and lower number of cases in the *S. aureus* herd. The results show that intervention strategies including interventions against contagious or opportunistic clinical and subclinical IMI can be highly cost-effective, but should be herd-specific.

KEY WORDS: dairy cattle, mastitis, control, simulation model

INTRODUCTION

Mastitis, or intramammary infection (IMI), causes considerable economic losses for many dairy cattle farms (e.g., Halasa et al., 2007). Costs for IMI arise from both treatment of cases and replacement of prematurely culled animals, as well as, indirectly, from production losses (e.g., Halasa et al., 2007). Production losses occur as a response to the inflammation, for both clinical

(Gröhn et al., 2004; Hertl et al., 2014) and subclinical mastitis (Halasa et al., 2009a; Hortet et al., 1999).

Consequently, there is a multitude of studies investigating the economic effects of clinical (e.g., Bar et al., 2008; Hagnestam-Nielsen and Østergaard, 2009) and subclinical IMI (e.g., Halasa et al., 2009b; Huijps et al., 2008). However, only few studies explicitly investigated the economic effects of intervention against clinical (Halasa, 2012) or subclinical IMI (e.g., van den Borne et al., 2010a; Steeneveld et al., 2007; Swinkels et al., 2005a), mainly focusing on treatment with antibiotics. Moreover, interventions against IMI are typically investigated separately for clinical or subclinical cases, albeit that clinical and subclinical IMI are a joint problem. Reducing the number of infected quarters through, e.g., treatment of subclinical cases should also lead to less flared up clinical cases (see, e.g., van den Borne et al., 2010a). In the case of contagious transmission, a good intervention strategy against clinical IMI can be expected to decrease IMI transmission (e.g., Steeneveld et al., 2011), thereby also leading to less subclinical cases. It is therefore not immediately visible how intervention strategies against subclinical or clinical IMI may interact. To our knowledge, there are no previous studies investigating the economic effects of interventions against subclinical and clinical IMI combined. Furthermore, the usual investigated intervention strategies are antibiotic treatments. Although culling could also be considered as an intervention against IMI, it is commonly only regarded as a consequence of IMI (Halasa and Hogeveen, 2018) and studied in the context of optimal replacement times (e.g., Cha et al., 2014; Heikkilä et al., 2012).

Consumer awareness regarding antimicrobial resistance and its connection with antibiotic usage in food animals is rising (Ruegg, 2003). Thus, while antibiotics are important to control and prevent IMI, prudent use of antibiotics is essential. A combined cow-specific approach of culling or treating animals with subclinical IMI may therefore be sensible. For instance, low producing cows could be culled instead of treated if they are diagnosed with contagious subclinical IMI. This would minimize the risk of pathogen spread to healthy herd mates and reduce additional antibiotics usage, while still improving herd health. Still, the economic effects of such a strategy must be investigated.

The aim of this study was to evaluate intervention strategies against subclinical IMI, combined with different intervention strategies against clinical IMI, caused by contagious or opportunistic (both contagious and environmental) IMI causing pathogens. For this purpose, two different Danish dairy herds with 200 cows including cow- and strain-specific IMI transmission were modelled. In one herd, IMI cases were mainly caused by contagious *Staphylococcus (S.) aureus*, while they were mainly caused by opportunistic *Streptococcus (St.) agalactiae* in the other herd. These pathogens were chosen as they are of major concern in Denmark. For these herds, several subclinical and clinical intervention strategies were combined and farm economics, antibiotic usage, culling, and epidemiological parameters were compared.

MATERIALS AND METHODS

Herd and Transmission Model

The MiCull (Mastitis-iCull) model version 3.0 was used in this study. The original MiCull version 1.0 was described in detail in Gussmann et al. (2018b), MiCull version 2.0 includes different intervention strategies against clinical IMI (Gussmann et al., unpublished data). The current version 3.0 additionally includes intervention strategies against subclinical IMI, as described below. The model was programmed and all simulations were run in the statistical computing software R version 3.2.2 "Fire Safety" (R Core Team, 2016). Figures were made using the package ggplot2 (Wickham, 2009).

HERD MODEL. The model is a stochastic mechanistic population model that simulates a Danish dairy herd with 200 cows in daily time steps (Gussmann et al., 2018b). All animals belong to one of five compartments (calves, heifers, lactating cows, dry cows, calving area), and move on to the next compartment after a stochastically determined number of days. Lactation (milk, protein, and fat) and somatic cell count (SCC) curves are estimated for all cows based on Græsbøll et al. (2016) and adjusted for IMI, i.e. increased SCC (Schepers et al., 1997; Wilson et al.,

1997) and decreased milk yield (Gröhn et al., 2004; Hortet et al., 1999). Once a month, milk yield and SCC are recorded, and the expected future average production (FAP) of a cow is estimated as described by Græsbøll et al. (2017). If a cow is treated with antibiotics, the milk is withdrawn and discarded during treatment and for six days afterwards. Feeding of lactating cows depends on the produced milk.

TRANSMISSION FRAMEWORK. The model simulates IMI spread in a dairy herd on quarter level (Gussmann et al., 2018b). It currently includes five pathogen strains: a contagious *S. aureus*, both a contagious and an environmental *St. uberis*, *St. agalactiae* with contagious and environmental elements at the same time (opportunistic), and environmental *Escherichia coli*. Heifers are exempt from dynamic transmission and have a probability to already be infected at calving. For lactating cows, the infection probability for every non-infected quarter is calculated every day. This probability depends on the active pathogen strains, the susceptibility, and, for contagious pathogens, on the number of infected quarters. Transmission of St. agalactiae has an environmental and a contagious part (Gussmann et al., 2018b). The susceptibility is relative to previously uninfected primiparous cows and is determined by risk factors such as parity or previous IMI (Zadoks et al., 2001). A newly infected quarter can appear as either a subclinical or a clinical case, depending on a pathogen-specific probability (Halasa et al., 2009b). Clinical quarters are treated with a 3-day intramammary treatment. After treatment ended, the quarter will either recover to susceptible with a cow-specific probability, or return to subclinical (Steeneveld et al., 2011). Subclinical quarters have daily pathogen-specific probabilities for spontaneous recovery (return to susceptible) or for flare up (become clinical).

Before a cow with a clinical IMI during lactation or a high SCC (200,000 or higher) at one of the last three monthly recordings is dried off, a test of a pooled milk sample by polymerase chain reaction (PCR) will be simulated (sensitivity and specificity are given in Table IV.1). If the test result is positive, the cow receives dry cow treatment, otherwise it will be dried off without dry cow treatment. New IMI and spontaneous recovery can occur during

Parameter	Description	Value		Reference
		Herd 1	Herd 2	
Transmission rate	Rate for susceptible quarters to enter infected state	0.0009	0.003	Fitted values
Probability of clinical state	Probability for a quarter to enter clinical state upon infection	0.17	0.01	See Table IV.S1
Flare up probability	Probability for a subclinical quarter to become clinical	0.0081	0.0005	See Table IV.S1
Spontaneous recovery probability	Probability for a subclinical quarter to become susceptible (without treatment)	0.0064	0.0022	See Table IV.S1
Recovery probability	Probability for a clinical quarter to become susceptible after treatment	0.4	0.7	See Table IV.S1
Test sensitivity	Test sensitivity for PCR (used at dry off)	0.908		Mahmmod et al. (2013)
Test specificity	Test specificity for PCR (used at dry off)	0.988		Mahmmod et al. (2013)
Probability to identify pathogen	Probability to identify causative pathogen by PCR	0.85		Taponen et al. (2009)

Table IV.1: Model parameters for the main causative pathogens (*S. aureus* in Herd 1 or *St. agalactiae* in Herd 2) during lactation

the whole dry period (Halasa et al., 2010). However, infection probabilities are simulated to be lower for cows with dry cow treatment, and they are independent of the number of infected quarters (Halasa et al., 2009c). Clinical cases (flared up and newly infected) can only occur in the first or last week of the dry period. If a cow becomes clinical in the first week, it will receive dry cow treatment.

CULLING. Cows are assessed for culling once a week, if the total number of cows in the herd exceeds the target count of 200 dairy animals (Gussmann et al., 2018b; Kirkeby et al., 2016). Parity, reproduction status, low milk yield, high SCC, and previous cases of clinical IMI are weighted, and the cows with the highest weights are culled. Involuntary culling, i.e. culling for other reasons such as, e.g., lameness, happens with a certain probability and is prioritized over voluntary culling (Kirkeby et al., 2016). Culling costs include the market value of a new heifer and the slaughter value of the culled cow (see Table IV.2).

Intervention Strategies for IMI

For this study, two different Danish dairy cattle herds were simulated. In both herds, the primary pathogen contained a contagious element, however, the transmission mode and param-

	Price	Reference
1 kg protein	5.8132	www.arla.dk, September 2017
1 kg fat	4.1519	www.arla.dk, September 2017
Handling of 1 kg milk	−0.01343	www.arla.dk, September 2017
Costs of a heifer	1000	Assumed market value
Slaughter value per cow	483	Kudahl et al. (2007)
Feeding		
Per calf per day	−0.0026	Kirkeby et al. (2016)
Per heifer/dry cow per day	−0.9311	Kirkeby et al. (2016)
Per energy corrected milk	−0.1947	Kirkeby et al. (2016)
Treatment (per day)	−11.10	Michael Farre (SEGES, Aarhus, Denmark, personal communication)
Opportunity cost (per case per day)	−6.66	Halasa et al. (2009b); Michael Farre (SEGES, Aarhus, Denmark, personal communication)
Dry cow treatment	−9.60	Michael Farre (SEGES, Aarhus, Denmark, personal communication)
Bacterial culture	−12	Michael Farre (SEGES, Aarhus, Denmark, personal communication)
PCR	−13.3	Michael Farre (SEGES, Aarhus, Denmark, personal communication)

Table IV.2: Prices in EUR used in the model to calculate income (positive values) and costs (negative values)

eters differed. In one herd, the majority of IMI cases were caused by purely contagious *S. aureus* (Herd 1), while opportunistic *St. agalactiae* (Herd 2) caused most subclinical cases in the second herd. Intervention measures included treatment, testing, and culling, and they were aimed at both clinical and subclinical cases. For the strategies, clinical and subclinical intervention measures were combined, with three baseline strategies (only clinical intervention measures). Results for subclinical intervention TestTreatCull (see below) are only shown in combination with clinical intervention Basic3.

CLINICAL IMI: INDIFFERENT TREATMENT FOR ALL CASES (BASIC3). In the default intervention, all clinical cases receive a 3-day intramammary treatment.

CLINICAL IMI: TESTING BEFORE TREATMENT (BEFORE50). A milk sample of every new clinical quarter is sent for testing by PCR. After one day, test results return and are used to calculate an expected recovery probability, which depends on the causative pathogen (85 % probability for correct identification, see Table IV.1), history of IMI, parity, days in milk (DIM), and SCC at the last milk recording (Steeneveld et al., 2011). For unidentified pathogens, recovery probability is a mean base cure probability

(see Table IV.1). Cows with a recovery probability below 50 % are culled, all other cases are treated.

CLINICAL IMI: CULLING WITH EXCEPTIONS (NOTCULLTOP). Similarly to the Before50 strategy, cows with a recovery probability below 75 % are culled. However, as described above, the FAP is calculated for all cows and those in the top 25 % according to FAP are always treated (i.e., these cows are not culled).

SUBCLINICAL IMI: TEST, TREAT AND CULL (TESTTREATCULL). In this strategy, cows with a high SCC (>200,000) in two consecutive milk recordings are tested by PCR. Test results return after one day and positive quarters receive 3-day intramammary treatment. After one month, quarters are re-tested and cows are culled, if the test is positive. Intervention against clinical IMI is Basic3.

SUBCLINICAL IMI: TREATMENT WITH EXCEPTIONS (CULLBOTTOM).
Cows with a high SCC in two consecutive milk recordings are tested by PCR and test results return after one day. Positively tested cows are culled if they are in the bottom 25 % according to FAP, otherwise the respective quarter is treated intramammary for three days. This strategy is combined with each of the intervention strategies against clinical IMI (CullBottom & Basic3, CullBottom & Before50, CullBottom & notCullTop).

SUBCLINICAL IMI: COW-SPECIFIC TREATMENT (TREATTOPLONGER).

Similarly to CullBottom, cows with a high SCC in two consecutive milk recordings are tested by PCR and test results return after one day. Positively tested cows are culled if they are in the bottom 25 % according to FAP, otherwise the respective quarter is treated intramammary. Treatment lasts for five days, if the cow is in the top 25 % according to FAP, or three days otherwise. This strategy is combined with each of the intervention strategies against clinical IMI (TreatTopLonger & Basic3, TreatTopLonger & Before50, TreatTopLonger & notCullTop).

Simulations and Model Output

We simulated the presented strategies for five years with a preceding five year burn-in period. The three clinical intervention strategies without added subclinical intervention serve as baseline strategies. 500 iterations per strategy insured stable results that described the effects of the strategies rather than the initial parameter values.

In the simulated five year period, the economic and epidemiological model outputs were collected: income from milk (depending on fat and protein prices, a fee for milk handling, and a bonus or penalty for the bulk tank SCC), IMI related costs (testing, lactational and dry cow treatment, opportunity costs, culling), other costs (feed, culling with a high SCC or history of IMI), number of subclinical cases (susceptible or clinical quarters entering subclinical state, including infected quarters of heifers at first calving), number of clinical cases (susceptible or subclinical quarters entering clinical state), number of treatment days (e.g., three treatment days for a 3-day treatment), and number of culled cows (culling as IMI intervention or with a high SCC or history of IMI). The gross income for the farm was calculated by subtracting the mentioned costs from the income from milk, while additional expenses (e.g., costs for other diseases, other costs related to cattle, buildings, and machinery) were not considered. Model output is presented as rounded median values of the annual arithmetic mean over five simulated years (including the 5th and 95th percentiles). IMI cases are counted on quarter level. Prices are given in Table IV.2.

Sensitivity analyses

Sensitivity analyses were performed for base cure probability after lactational treatment; fat and protein prices in Denmark in 2017 (`www.arla.dk/om-arla/ejere/arlapris/2017`); and culling costs (Table IV.3). For *St. agalactiae* (Herd 2), an additional sensitivity analysis for the environmental part in transmission was conducted (Table IV.3).

Sensitivity analysis	Values				
Base cure probability (Herd 1)	0.2	0.4*	0.6		
Base cure probability (Herd 2)	0.5	0.7*	0.8		
Environmental part in *St. agalactiae* transmission	0.1	0.25	0.5	0.75	0,9
Milk price					
Price for 1 kg protein / 1 kg fat in EUR	5.25/3.75	5.51/3.94	5.66/4.04	5.81/4.15*	5.92/4.23
Additional costs for culling in EUR	0*	250	500	1,000	

Table IV.3: Values used in the sensitivity analyses, default values (Table III.1 and III.2) are marked by *

RESULTS

An overview of all results can be found in Table IV.4, with input values for sensitivity analyses in Table IV.3.

In Herd 1, where the main problem was S. aureus IMI, the baseline strategy Basic3 yielded a median yearly income of €187,666, with a median of 42 clinical and of 136 subclinical cases per year. There were 123 treatment days per year and 16 cows per year culled in relation to IMI (median values). The other two baseline strategies Before50 and notCullTop led to a higher median yearly income (about €197,000) and lower median yearly number of cases (clinical and subclinical), with substantially less treatment days and more cows culled in relation to IMI (Table IV.4). All strategies with intervention against subclinical IMI could further improve the median yearly income and reduce the number of cases.

Combined with Basic3, the strategies TestTreatCull, CullBottom, and TreatTopLonger led to comparable yearly numbers of clinical (27 in median) and subclinical (120–122 in median) cases. Among these three strategies, TestTreatCull yielded the lowest median yearly income (€198,418) with the other two strategies yielding about €2,000 (CullBottom) and €2,500 (TreatTopLonger) more per year (median values). The median yearly numbers of treatment days were higher than in the baseline strategies, ranging from 149 (CullBottom) to 193 treatment days (TestTreatCull). The median yearly number of culled cows was 23 with strategy TestTreatCull and 45 and 43 with strategies CullBottom and TreatTopLonger, respectively.

When the strategies CullBottom and TreatTopLonger were combined with one of the other two baseline strategies Before50 and notCullTop, the median yearly income was between €204,000

Strategy	Gross income		Clinical IMI cases		Subclinical IMI cases		Treatment days		Culled cows	
Herd 1 (*S. aureus*)										
Basic3	187,666	(173,363; 202,147)	42	(33; 51)	136	(121; 161)	123	(95; 151)	16	(12; 20)
TestTreatCull[1]	198,418	(185,806; 213,398)	27	(11; 33)	120	(75; 133)	193	(118; 226)	23	(10; 29)
CullBottom[1]	200,491	(187,248; 215,304)	27	(11; 33)	122	(74; 135)	149	(77; 177)	45	(28; 52)
TreatTopLonger[1]	201,029	(188,613; 214,898)	27	(11; 33)	122	(74; 135)	158	(85; 189)	43	(30; 52)
Before50	196,995	(181,427; 211,492)	30	(15; 38)	111	(75; 130)	47	(36; 64)	24	(10; 29)
CullBottom & Before50[2]	206,342	(192,801; 218,478)	14	(10; 27)	83	(71; 120)	86	(67; 115)	34	(26; 51)
TreatTopLonger & Before50[2]	205,975	(193,335; 218,272)	14	(10; 27)	84	(71; 118)	92	(71; 127)	35	(26; 51)
notCullTop	196,704	(182,197; 213,318)	32	(14; 39)	113	(72; 127)	32	(12; 43)	29	(16; 34)
CullBottom & notCullTop[3]	205,383	(192,057; 216,535)	16	(10; 30)	87	(71; 123)	76	(48; 108)	40	(30; 54)
TreatTopLonger & notCUllTop[3]	204,240	(191,357; 217,887)	15	(10; 29)	85	(71; 122)	79	(54; 121)	39	(29; 53)
Herd 2 (*S. agalactiae*)										
Basic3	155,170	(135,863; 176,596)	21	(17; 30)	130	(110; 164)	62	(50; 87)	22	(17; 27)
TestTreatCull[1]	200,176	(188,232; 211,562)	13	(11; 16)	96	(86; 107)	149	(130; 169)	17	(14; 21)
CullBottom[1]	200,333	(188,264; 214,044)	13	(10; 16)	97	(87; 107)	107	(92; 124)	39	(33; 45)
TreatTopLonger[1]	200,494	(188,813; 211,936)	13	(10; 16)	96	(86; 107)	115	(97; 136)	38	(31; 46)
Before50	157,690	(137,244; 178,725)	21	(17; 30)	126	(108; 162)	50	(40; 73)	24	(19; 29)
CullBottom & Before50[2]	200,286	(187,645; 212,006)	13	(10; 16)	96	(87; 107)	104	(88; 120)	39	(34; 47)
TreatTopLonger & Before50[2]	200,367	(189,299; 211,525)	13	(10; 16)	96	(87; 106)	112	(96; 132)	39	(34; 45)
notCullTop	158,885	(138,046; 181,690)	20	(16; 25)	121	(105; 145)	18	(12; 25)	31	(25; 37)
CullBottom & notCullTop[3]	199,748	(188,168; 211,576)	13	(10; 16)	94	(85; 104)	82	(66; 98)	42	(35; 48)
TreatTopLonger & notCullTop[3]	199,649	(187,233; 212,443)	13	(10; 15)	94	(84; 103)	89	(73; 106)	42	(35; 48)

[1]Intervention strategies combined with Basic3
[1]Intervention strategies combined with Before50
[1]Intervention strategies combined with notCullTop

Table IV.4: Median model output (with 5th and 95th percentiles) of 500 iterations for a herd with 200 dairy cows, simulated over 5 years: income in € (income from milk minus costs related to IMI and for feeding), number of clinical IMI cases, number of subclinical IMI cases, number of treatment days, and number of culled cows (due to IMI intervention, or with a high SCC or history of IMI).

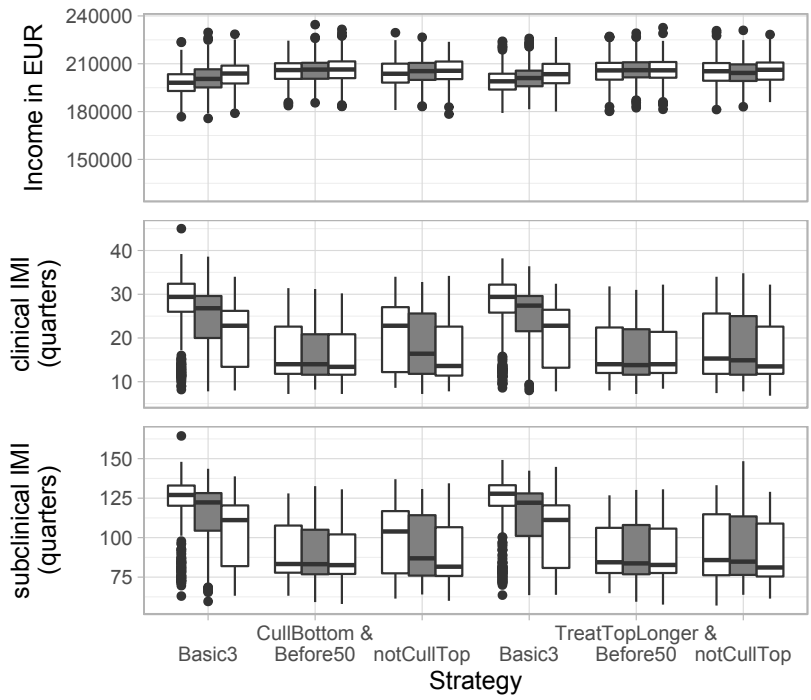

Figure IV.1: Results of the sensitivity analysis for the base cure probabilities in Herd 1 (*S. aureus*). The box plots show the mean annual income (income from milk minus costs for feed, culling, dry cow treatment, and IMI intervention), mean annual number of clinical IMI cases, and of subclinical IMI cases. Results with default values are marked in gray.

and €206,000. Median yearly numbers of clinical cases ranged from 14 to 16 and from 83 to 87 for subclinical cases. There were more treatment days (around 40) than in the respective baseline strategies (median yearly numbers), but less than with Basic3. The median yearly number of cows culled in relation to IMI was higher than that in the baseline strategies, but lower than in the CullBottom and TreatTopLonger strategies when combined with Basic3.

In Herd 2, where *St. agalactiae* IMI was the main problem, the median yearly income with the baseline strategies was much lower than in Herd 1 (€155,170 with Basic3). The median yearly number of clinical cases was 20 (notCullTop) or 21, and the median yearly number of subclinical cases was between 121 and

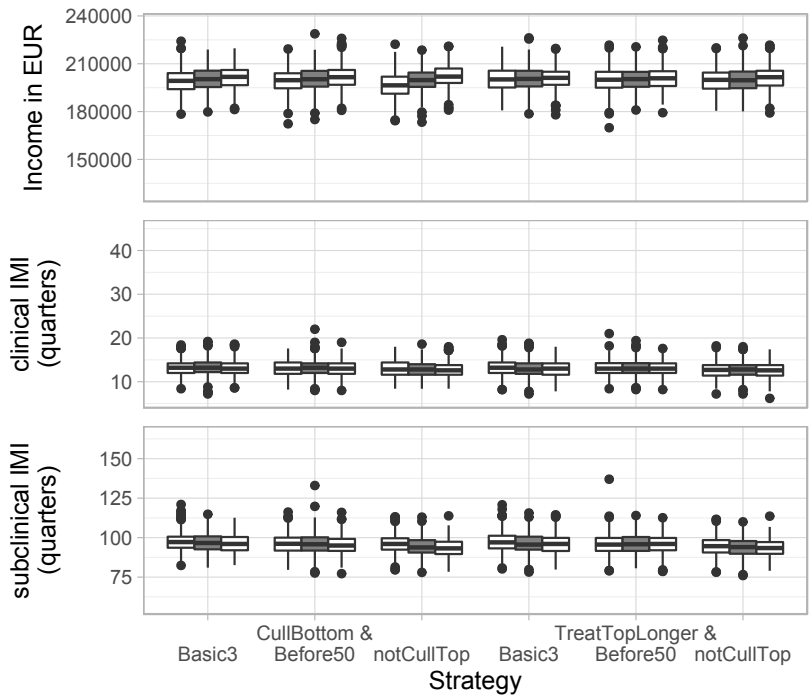

Figure IV.2: Results of the sensitivity analysis for the base cure probabilities in Herd 2 (*St. agalactiae*). The box plots show the mean annual income (income from milk minus costs for feed, culling, dry cow treatment, and IMI intervention), mean annual number of clinical IMI cases, and of subclinical IMI cases. Results with default values are marked in gray.

130. There were 62 treatment days with Basic3 and 18 treatment days with notCullTop (median yearly values). Conversely, a median number of 22 (Basic3) and 31 (notCullTop) cows were culled per year in relation to IMI.

With all other strategies, the median yearly income was around €200,000, there were a median of 13 clinical cases and of 94 to 97 subclinical cases per year. The median yearly number of treatment days was higher than in the baseline strategies, ranging from 149 (TestTreatCull) down to 82 (CullBottom & notCullTop). Similarly, more cows were culled in relation to IMI (between 38 and 42 in median), with the exception of strategy TestTreatCull, where the number was reduced to 17.

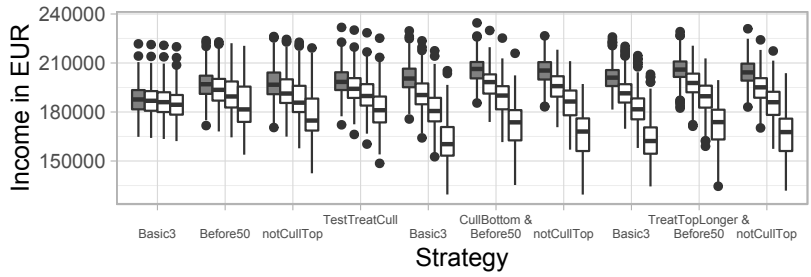

Figure IV.3: Results of the sensitivity analysis for culling prices in Herd 1. The box plots show the mean annual income (income from milk minus costs for feed, culling, dry cow treatment, and IMI intervention). Results with default values are marked in gray.

Sensitivity analysis for the base cure probability after lactational treatment showed that the yearly income increased and the number of cases decreased (both clinical and subclinical) with increasing cure probability. In Herd 1 (*S. aureus*), this trend was most visible when the clinical intervention was Basic3 and least visible when it was Before50 (Figure IV.1). In Herd 2 (*St. agalactiae*), the trend was less visible, but more dependent on the subclinical than the clinical intervention (Figure IV.2).

An increase of the environmental part of *St. agalactiae* transmission led to a slight decrease in income and increase in the number of subclinical cases (results not shown).

Sensitivity analysis on the fat and protein prices showed a high dependency of the yearly income on the milk price for all strategies, with median values ranging from €109,000 to €187,000 for baseline strategy Basic3 in Herd 1. Differences between strategies were similar, independent of the milk price (results not shown).

Variation of culling costs showed a decrease in income for increased costs. Reduction in the yearly income was higher in strategies where more cows were culled (Figure IV.3).

DISCUSSION

The objective of this study was to evaluate different combinations of intervention strategies against clinical and subclinical IMI of contagious or opportunistic origin, as these are of major concern in Danish dairy cattle herds. For that purpose, three clinical and three subclinical strategies were combined. Altogether, ten different strategies were presented for two different herds, one with an *S. aureus* problem (Herd 1) and another with a *St. agalactiae* problem (Herd 2). Three of those were baseline strategies, i.e. strategies without intervention against subclinical IMI. In all strategies, clinical cases were treated with intramammary antibiotic injections for three days or culled, though there was no reactive culling in the baseline strategy Basic3. Interventions against subclinical mastitis consisted of testing, 3-day intramammary treatment, and reactive culling of persistently infected cows (TestTreatCull). The other subclinical strategies reflected cow-specific control measures. Low producing cows could be subjected to reactive culling instead of treatment (3 CullBottom and 3 TreatTopLonger strategies), and high producing cows could be treated for five instead of three days, if tested positive for subclinical IMI (all TreatTopLonger strategies).

Model results showed that adding intervention measures against subclinical IMI to a clinical intervention strategy led to a higher yearly income and both less clinical and less subclinical cases (Table IV.4). The increase in yearly income was especially noticeable in Herd 2, where most subclinical cases were caused by *St. agalactiae*. This is not surprising, as *St. agalactiae* is mostly associated with subclinical IMI (Keefe, 1997), so intervention against subclinical IMI could be expected to be more effective than intervention against clinical IMI. These findings are in general consistent with earlier studies that have shown that interventions against contagious subclinical IMI could be cost-effective (e.g., van den Borne et al., 2010a). However, our results suggest additionally, that altering clinical intervention on top of adding subclinical interventions could in some cases lead to an even higher yearly income, while further reducing the number of IMI cases (Herd 1, Table IV.4). If clinical cases are rare, as in Herd 2, the clinical intervention strategy does not seem to

be important, if an intervention strategy against subclinical IMI is in place (Herd 2, Table IV.4). This illustrates, that it is important to make herd-specific decisions, taking the clinical manifestation of the causative pathogen into account when choosing an intervention strategy in the herd. Schukken et al. (2012) also pointed out that herd-specific decisions are important for control and prevention of IMI.

Although intervention against subclinical IMI lost a bit in effectiveness when the environmental part of transmission of opportunistic *St. agalactiae* was higher in sensitivity analysis, the differences between Herd 1 and 2 were more pronounced. The difference between the two herds was also visible in the sensitivity analysis on the base cure probability. In Herd 1, sensitivity to the cure probability seemed to be more dependent on the clinical part of the intervention strategy (Figure IV.1). Contrarily, changing the subclinical part of the intervention strategy had a greater influence on results in Herd 2 (Figure IV.2). This further emphasizes the importance of herd-specific decisions.

In both herds, the most cost-effective strategies included intervention measures against subclinical IMI and there were several strategies that seemed similarly cost-effective in terms of yearly income and number of IMI cases. The difference between these strategies could be seen in the number of treatment days and culled cows. Generally, changing the clinical intervention could reduce antibiotic usage at the cost of an increased number of culled cows (compare, e.g., CullBottom & Before50 and CullBottom & notCullTop, Table IV.4). The same trend could be seen when comparing strategy TestTreatCull with one of the other subclinical strategies. Changing subclinical intervention from CullBottom to TreatTopLonger did not seem to affect the number of culled cows, but slightly increased treatment days. Strategies including TreatTopLonger may therefore not be ideal. Nevertheless, the results show that cost-effective strategies still come with a certain price: increased antibiotics usage or reduced longevity. The farmer will have to decide which kind of costs he or she can best justify for their farm. A possibility to partly avoid this particular dilemma could be to reduce IMI transmission rates in the herd through rigorous hygienic or biosecurity measures (Lam et al., 1996). This would lead to a reduced IMI incidence, while

also reducing both treatment days and the number of cows culled in relation to IMI (results not shown). However, there are few studies investigating cost-effectiveness of hygienic measures (e.g., Huijps et al., 2010), so it remains unclear if the increase in income from milk would compensate for the costs of implementing a comprehensive hygiene strategy.

In this study, we did not change prices over time. That may be unrealistic, but it simplifies comparison of different intervention strategies, as extra noise is removed. Sensitivity analysis of culling costs and milk prices showed that both have a substantial influence on the yearly income (Figure IV.3). However, the implications differ; while an increased milk price led to a higher income in all strategies, the extent to which higher culling costs lowered the yearly income depended on the intervention strategy. Therefore, culling costs should be taken into account upon choosing an intervention strategy, too.

The presented strategies included strict culling rules and led to a high number of culled cows. However, the culling rules for subclinical IMI concentrated on removing persistently infected or low producing cows. As a previous study showed that in some Danish herds a high milk production could be a determinant for antimicrobial treatment (Gussmann et al., 2018a), it does not seem so farfetched that farmers may be willing to consider culling low producing cows. This would also conform to a study by Vaarst et al. (2006), where farmers were willing to adopt culling strategies that fit into their goal for the herd. The strict culling rules also affect the usual culling procedure in the model, as we simulated a closed herd where replacement heifers had to be reared on-herd. In a real herd, where other, non-voluntary culling occurs, e.g. due to other diseases, a strict culling strategy in relation to IMI may be challenging to implement. Furthermore, there is an ethical aspect to consider: strict culling rules may reduce longevity and thus impede animal welfare (Bruijnis et al., 2013). In this study, however, we focussed on the economic and epidemiological effects of IMI interventions, and we leave it to future studies to investigate how general culling dynamics are influenced by strict culling rules for IMI.

It should be kept in mind, that this study used a modelling approach to investigate different intervention strategies. The

results depend on the model parameters, e.g., transmission and cure rates, and on the modelled herd structures. These are likely to differ to a certain degree in real herds. However, while a field study to validate the results would be ideal, it would also be costly and the trends found in the results are clear. The differences in cost-efficiency could be explained and were far from marginal, allowing the strong belief that the results of the model are trustworthy.

CONCLUSIONS

We investigated different combinations of cow-specific intervention strategies against contagious or opportunistic clinical IMI and subclinical IMI in two situations; in a herd with *S. aureus* as the main causative pathogen, and in another herd with *St. agalactiae* as the main causative pathogen. Intervention measures generally led to an increased number of culled animals or higher intake of antibiotics. We demonstrated that intervention strategies against both subclinical and clinical IMI, including cow-specific treatment and culling decisions, could reduce IMI incidence and thereby increase the farm's yearly income in the long term. In addition, the optimal intervention strategy was dependent on the main causative pathogen within the herd, illustrating that control of IMI must be herd-specific.

ACKNOWLEDGEMENTS

This study is part of the STOPMAST project, funded by the Danish Milk Levy Foundation (Mælkeafgiftsfonden), and part of the EMCo-Mast project, funded by the Green Development and Demonstration Program (GUDP) under the Danish Directorate for Food, Fisheries and Agriculture, grant no. 34009-15-0918.

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SUPPLEMENTARY MATERIAL

Parameter	Description	Value (lactation)	Value (dry period)	Reference (value lactation)
Transmission rate	Rate for susceptible quarters to enter infected state			
	S. aureus	0.00004	0.0009	Fitted value
	S. agalactiae	0.0007	0.0001	Fitted value
	S. uberis (contagious)	0.0002	0.00001	Fitted value
	S. uberis (environmental)	0.000002	0.0000001	Fitted value
	E. coli	0.000001	0.000001	Barkema et al. (1998)
Probability of clinical state	Probability for a quarter to enter clinical state upon infection			
	S. aureus	0.17	0.1	Swinkels et al. (2005a)
	S. agalactiae	0.01	0.1	Barkema et al. (1998) and Swinkels et al. (2005a)
	S. uberis (contagious)	0.32	0.1	Zadoks et al. (2003)
	S. uberis (environmental)	0.32	0.1	Zadoks et al. (2003)
	E. coli	0.85	0.1	Hogan and Smith (2003)
Flare up probability	Probability for a subclinical quarter to become clinical			
	S. aureus	0.0081	0.006	Swinkels et al. (2005a)
	S. agalactiae	0.0005	0.0005	Barkema et al. (1998) and Swinkels et al. (2005a)
	S. uberis (contagious)	0.0068	0.004	Swinkels et al. (2005b)
	S. uberis (environmental)	0.0068	0.004	Swinkels et al. (2005b)
	E. coli	0.0035	0.0035	Döpfer et al. (1999)
Spontaneous recovery probability	Probability for a subclinical quarter to become susceptible (without treatment)			
	S. aureus	0.0064	0.0079	van den Borne et al. (2010b)
	S. agalactiae	0.0023	0.0086	Leelahapongsathon et al. (2016)
	S. uberis (contagious)	0.0143	0.0086	van den Borne et al. (2010b)
	S. uberis (environmental)	0.0143	0.0086	van den Borne et al. (2010b)
	E. coli	0.0221	0.0221	van den Borne et al. (2010b)
Recovery probability	Probability for a clinical quarter to become susceptible after treatment or dry cow treatment			
	S. aureus	0.4	0.77	Steeneveld et al. (2011)
	S. agalactiae	0.7	0.89	Steeneveld et al. (2011)
	S. uberis (contagious)	0.7	0.89	Steeneveld et al. (2011)
	S. uberis (environmental)	0.7	0.89	Steeneveld et al. (2011)
	E. coli	0.8	0.9	Steeneveld et al. (2011)

Table IV.S1: Model parameters for all secondary pathogens during lactation and dry off, references are given for values during lactation. Dry period values are taken from Halasa et al. (2010).

5 GENERAL DISCUSSION

5.1 DATA ANALYSIS

In the first manuscript (Section 3.2), we showed that data from the Danish Cattle Database can be used to categorise herds into different groups regarding use of antibiotic treatment for udder health. Prominent determinants for treatment were, e.g., health indicators (including diagnostics), age, or milk production.

In addition to the investigated factors, other non-investigated factors could predict treatment, maybe even better than in our analysis. For instance, Jansen et al. (2009) found that farmer attitudes such as a perceived lack of control of mastitis could explain a large part of the variation within subclinical or clinical mastitis incidence. Therefore, it does not seem farfetched that farmer attitudes might also influence antibiotic treatment. In Denmark, antibiotic treatment must be prescribed by a veterinarian. Considering that, another influential factor for antibiotic treatment could be the herd veterinarian or the existence of a herd health programme (Lind et al., 2012). In contrast to farmer attitudes, which are not registered in a database, records of the responsible herd veterinarinan should exist and could therefore, theoretically, be considered in the analysis without too much effort. However, in the data made available to us, information about the veterinarian was not included.

In general, the disease recording data for clinical mastitis seem to be of good quality in Denmark (Wolff et al., 2012). However, the Danish Cattle Database is not complete, especially where it concerns non-mandatory data, e.g., dry off dates. These have to be considered as a possible source of bias, when analyses are conducted with this data. If herds have to be excluded from analyses because their data are incomplete, results may not be applicable for the whole target population. It is therefore important to realise which population the study population can

be assumed to sufficiently represent. Within a herd, incomplete data are harder to recognise and deal with. If registrations are systematically missing, wrong, or unavailable, results are likely falsified. Randomly missing data on the other hand should still produce usable results. Unfortunately, this can rarely be determined from the database alone, so a certain trust in the register data is required.

On top of missing data, registration errors can occur and lead to inconsistent data and further bias. An example of inconsistent data was a clinical registration for a cow 8236 days (around 22.5 years) after calving. This was most likely caused by a registration error for the date or the cow identity. Registration errors can in many cases be detected, although they cannot be easily corrected without further information. Dealing with these kinds of error is part of data curation.

However, inconsistency can also be caused by subjective assessment. Disease recording allows registration of cases as "Yverbetændelse, subklinisk" (subclinical inflammation of the udder), "Yverbetændelse" (inflammation of the udder), or "Yverbetændelse, akut" (acute inflammation of the udder), but also of mastitis pathogens. Different persons may have different habits or opinions about when a case classifies as acute, or when to register the causative pathogen instead of a subclinical case. This is one of the reasons why we did not differentiate between registrations in Manuscript I.

Despite errors and potential bias, register data are a valuable source of information, as they are recorded on a regular basis and include a large quantity of registrations covering a substantial proportion of the population. Obtaining this information via field studies would most likely be a logistically demanding and very costly challenge. The value of register data should therefore not be underestimated. Instead, the data should be constantly assessed, understood, curated, and improved. Most importantly, results of analyses based on register data should be interpreted with caution, bearing the potential bias in mind.

5.2 MODELLING

5.2.1 Implications across manuscripts

In Manuscript II (Section 4.3), the model was introduced and discussed. One of the results was that lower prevalences generally led to higher per-case costs (Section 4.2). This means that an individual IMI case was of less economic consequence if there were more cases in total. Since the goal is identification of cost-effective intervention measures, it may be better to look at the total income and costs, instead of comparing costs on a per-case basis.

Therefore, the economic output was presented as a net income in the following Manuscripts III (Section 4.4) and IV (Section 4.5). In these manuscripts, different intervention strategies against mastitis were investigated and compared. The investigated interventions took determinants for antimicrobial treatment (or aspects thereof) into account based on information found in Manuscript I (see above and Section 3.2): testing before treatment (diagnostics), considering milk production, or adjusting decision making for primiparous cows (age). Results for the latter were not presented, as there were no conspicuous changes compared to strategies where decisions were not adjusted.

However, while milk production was identified as a determinant for antimicrobial treatment in Manuscript I, use of milk yield as a determining factor in a treatment strategy was not specifically cost-effective (see strategy "Longer" in Manuscript III). When used as a determining factor for reactive culling in an intervention strategy, on the other hand, it could improve cost-effectiveness (strategy "notCullTop" in Manuscript III and "CullBottom" in Manuscript IV). For farmers who already use milk yield as a determinant for treatment, it may not be such a big step to switch to one of these strategies, provided that they are willing to consider strategies with reactive culling. Other farmers may be willing to consider these strategies because they believe in IMI diagnostics, or if their preferred mastitis management strategy can also be incorporated. Consequently, intervention strategies and their communication should be customised for the respective farmer (Jansen and Lam, 2012; Ritter et al., 2017).

5.2.2 Modelling IMI and IMI interventions

While the model is sensitive to changes in all transmission parameters, changes in the transmission rate seemed to be most effective and consistent in altering pathogen occurrence. The transmission framework, including opportunistic transmission, and the need for accurate estimates of transmission parameters were discussed in Manuscript II (Section 4.3). In the following two Manuscripts III (Section 4.4) and IV (Section 4.5), intervention strategies against clinical and subclinical IMI were investigated. We found that nearly every intervention strategy was more cost-effective than the standard three day treatment and that adding subclinical intervention to a clinical intervention strategy could further improve cost-efficiency. Depending on the main causative pathogen, a different combination of clinical and subclinical interventions was the preferable option.

Cow-specific interventions and reactive culling led to cost-effective strategies at the cost of increased antibiotics usage or culling. Increased use of antimicrobials may hamper societal acceptance of a strategy, as consumers are increasingly aware of antibiotics usage in food production and antimicrobial resistance (Ruegg, 2003). Similarly, increased culling may create negative impressions about the dairy industry, as longevity is linked to animal welfare (Bruijnis et al., 2013). Thus, ethics of targeted culling must be considered in practice. Decreasing mastitis incidence by improving hygiene would circumvent these problems (see Section 4.4). However, seeing that farmers consider extra labour as one of the most disturbing aspects of mastitis (Kuiper et al., 2005), they may not be eager to dedicate more time to a more thorough and rigorous daily hygiene regimen.

Another important point regarding hygiene was raised in Manuscript III: a comprehensive hygiene strategy would probably be most effective from an epidemiological point of view, but the cost-effectiveness of such a strategy is unclear. Why is hygiene different from the other intervention strategies, where cost-effectiveness was investigated by the modelling approach? Culling of infected animals is not directly connected to the transmission framework, but antibiotic treatment of IMI causes changes in the framework by increasing the cure rates (see Section 2.1.2).

Studies providing information about cure after treatment are therefore necessary (e.g., Steeneveld et al., 2011). Hygiene similarly intervenes directly in the transmission framework by reducing the transmission rate (see Section 2.1.2). Hence, parameter estimates regarding hygiene are needed for implementation in the model. While some studies about costs and efficacy of hygienic measures exist (e.g., Huijps et al., 2010), little is known about how different hygienic measures complement each other. Is their efficacy additive or are some measures more or less efficient than one would expect when combined? We could have compared single hygiene measures against different intervention strategies in Manuscript III. However, the focus of the manuscript was on intervention against clinical IMI in general, so we decided to emulate a comprehensive hygiene strategy by reducing the transmission rate and only compare effectiveness in this case.

Besides the intervention strategies introduced in Chapter 4, we also tested other strategies. These were not shown, because their results were not better than those of the presented strategies. There are also further additions that could have been considered in the model regarding the implemented options for intervention, e.g., selection strategies for dry cow treatment. At the moment, the model includes three options for selective DCT; cows can be selected for testing if they fulfill one or both of the following conditions: if they had an elevated SCC in the current, last, or second to last month; or if they had a clinical IMI during the lactation. Further options could also take the elevation level of the SCC or cow factors into account.

It is important to note that, regarding both clinical and subclinical intervention strategies, we focused on pathogens with contagious transmission (*S. aureus*) or transmission with contagious elements (*S. agalactiae*). The reason for this choice was that these pathogens depend on the number of infected quarters. Controlling this number by intervention measures was expected to inhibit spread of infection to healthy herd mates. Our results showed that taking action to clear infection could prevent new cases and milk loss. In environmental transmission, such a clear effect would not be expected. For a similar reason, *S. agalactiae* was only investigated in Manuscript IV, as it causes foremost subclinical IMI.

5.2.3 Model choice

The MiCull model is a mechanistic, stochastic model. That means that the assumed inner mechanisms of a dairy herd and IMI transmission and intervention are modelled in a stochastic manner. In that sense, models including IMI transmission are always mechanistic. Another type of model often used for mastitis are dynamic programming models. Dynamic programming models describe the modelled system, in this case a dairy cow, as a set of possibly hierarchical state variables with a discrete number of states (Bellman, 1957). At any time step, the states can change, depending on the assumed transition probabilities. The goal is to optimise a given objective function (net present value of a cow, Bar et al., 2008b; Cha et al., 2014). Considering their structure, these kinds of models are particularly suited for optimisation problems (Cha et al., 2014) or to investigate costs per case or cow (Bar et al., 2008b). As we were not only interested in herd economics, but also epidemiological effects, we chose the mechanistic approach.

This allowed us to test and compare different intervention strategies against clinical and subclinical IMI without conducting field studies. The limitations of this approach lie in the need to understand the underlying structures and their proper parameterisation. In the absence of Danish parameter estimates, literature values from studies conducted in other countries had to be used. If parameter estimates are not available at all as, e.g., for a comprehensive hygiene strategy, cost-effectiveness cannot be investigated. Missing quantitative knowledge is also the reason why not all known risk factors could be included in the model. Milk production level has for example been identified as a risk factor (e.g., Koeck et al., 2014), but for modelling purposes, a quantification is necessary. Similarly, to properly represent strain-specific characteristics for all strains, e.g., cure after treatment (van den Borne et al., 2010b), strain-specific parameter estimates are required. In the MiCull model, two strains of *S. uberis* are included. However, they differ only in their transmission modes and not in the other parameters, as the necessary parameter estimates are missing. They were added regardless to showcase strain-specificity of the model.

Nevertheless, as described in Chapter 4, the MiCull model complies with the requirements given in Section 2.3 (see also Figure 3), given that parameter estimates are available. Compared to the other models introduced in Section 2.2.2, it models IMI on quarter level and thus allows up to four different pathogens per cow. Transmission is strain-specific and allows one of three different transmission modes per strain. Pathogen-specific effects on production are modelled for both clinical and subclinical IMI. The model includes cow-specific infection and cure of clinical IMI. Furthermore, IMI is also modelled during the dry period. Finally, different options for DCT and intervention strategies against clinical and subclinical IMI are available, including cow-specific intervention and reactive culling. The model is therefore *strain-*, *cow-*, and *herd-specific*.

Finally, it should be remembered that at this stage, the model is still a scientific decision support tool. That is, the results obtained by the model should be seen as indications for possible cost-effective intervention strategies. Models are only as good as their input allows. In Manuscripts III and IV, a rigorous use of diagnostics and monthly monitoring of SCC were assumed. Farmers may, for one reason or another, not always strictly adhere to the strategy. This or other, unforeseen factors might influence the outcome in reality, so field testing of the suggested strategies to validate the model results would be ideal. According to Ritter et al. (2017), practical evidence would also help with motivating farmers to adopt management changes.

6 CONCLUSIONS

This thesis used a modelling approach to investigate costs and efficacy of different intervention strategies against IMI. The results showed that control of mastitis can be improved in cost-effectiveness by cow- and herd-specific strategies that include reactive culling and measures against subclinical IMI. Based on the research questions in Section 2.3, the following conclusions could be reached.

RQ1A Data from the Danish Cattle Database could be used to find determinants for antimicrobial treatment for udder health. Based on how much each factor contributes, herds could be classified into three different groups. These groups represented herds with resembling treatment practices.

RQ1B For lactational treatments, the three groups were characterised by age and diagnostics based treatment, production based treatment, and treatment in connection with culling. For DCT, age and diagnostic based treatment were separate groups with production based treatment as the third group.

RQ2 The developed model was sensitive to changes in all transmission parameters. However, changes in the transmission rate seemed to be most influential and consistent in altering pathogen occurence, followed by spontaneous recovery probability.

RQ3 Nearly every proposed intervention strategy resulted in a lower IMI incidence than a default three day intramammary treatment for all clinical cases without causing economic losses. Cow-specific intervention strategies that included testing and reactive culling led to better results.

RQ4 Adding any subclinical intervention measure could further improve an intervention strategy by another reduction of IMI incidence and increase in income. Additional subclinical intervention measures led to an increase in antibiotic usage and culling.

RQ5 There was not a unique best intervention strategy against IMI. Choice of strategy rather depended on the herd and the present causative pathogens. The optimal strategy would also change depending on the concessions the farmer is willing to make regarding costs in the form of antibiotic usage and culling of cows. However, the optimal intervention strategy in terms of pure cost-effectiveness always involved testing and cow-specific treatment or reactive culling decisions for both clinical and subclinical IMI.

7 PERSPECTIVES

The methods used for data analysis in Chapter 3 and the model presented in Chapter 4 can be used or expanded in the future.

Determinants for antibiotic treatment may change over time and so rerunning the analysis once in a while would help with monitoring changes in treatment behaviour. It may, in particular, be interesting so see if the determinants changed after the milk quota system was abolished in 2015. As mentioned in the discussion in Chapter 5, the herd veterinarian could also be included in future analyses. Furthermore, a similar analysis could be run to find determinants for culling.

Regarding the model, there are various possibilities for future studies. In Chapter 5, investigating effects of different hygiene measures or different selection strategies for dry cow treatment were discussed. As the results in this thesis have shown that intervention strategies should be herd-specific and herd sizes in Denmark are increasing, it may also be interesting to investigate how cost-effectiveness of intervention strategies changes with herd size. A less rigorous use of diagnostics and delayed treatment or culling decisions could be investigated to account for a more erratic component in human behaviour. It could also be interesting to consider and compare different SCC thresholds when selecting cows for testing for subclinical IMI.

Moreover, there are several factors that were not addressed in this thesis that could be included in the model in the future, for instance, drying off of single quarters, cow-specific cure after treatment of subclinical cases, or shedding patterns. The current model only includes a rudimentary implementation of heifer mastitis, this could also be expanded. Finally, a field study to validate some of the model results could be conducted.

APPENDIX

Parameter	Description	Pathogen	Value	Reference
Infection probability	Probability that a heifer will be infected with the pathogen at first calving	*S. aureus*	0.0171	De Vliegher et al. (2012)
		S. agalactiae	0.0188	De Vliegher et al. (2012)
		S. uberis (contagious)	0.0747	De Vliegher et al. (2012)
		S. uberis (environmental)	0.0747	De Vliegher et al. (2012)
		E. coli	0.0747	De Vliegher et al. (2012)

Table 2: Missing values from Table II.2. Values are means of the values for quarter level samples reported in Table 1 (De Vliegher et al., 2012).

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